ISSN (online): 2250-141X
Vol. 1 Issue 2, December 2011

Study of Cytotoxic and Antimitotic Activities of *Solanum nigrum* by Using *Allium* cepa Root Tip Assay and Cancer Chemo preventive Activity Using MCF-7- Human Mammary Gland Breast Adenocarcinoma Cell Lines

A.Thenmozhi[1], A.Nagalakshmi[2], Dr.U.S Mahadeva Rao[3*]

[1]Assistant Professor, PG Department of Biochemistry, SRM Arts and Science College, Chennai, India.
[2]Assistant Professor, PG Department of Biochemistry, DG Vaishnav College, Chennai, India.
[3]Associate Professor, Department of Biochem., Faculty of Medicine and Health Science, Universiti Sultan Zainal Abidin, Malaysia.

Corresponding Author: Dr.U.S.Mahadeva Rao.

ABSTRACT

Objective: *In the present study we have utilized the Allium cepa root tip meristem model to evaluate the cytotoxic and antimitotic activities of Solanum nigrum. The studies were extended to human cells using MCF-7- Human mammary gland breast adenocarcinoma cell lines.* ***Methods:*** *Preliminary antimitotic screening was done using Allium cepa root tip assay. The herbal powder obtained from plant part-dry leaves were extracted with various solvents. The antimitotic activity was analysed using Allium cepa root meristematic cells. Experiments were carried out with incorporation of folic acid in the extract. Folic acid inhibited the antimitotic activity of S.nigrum extract.* ***Findings:*** *The results obtained were compared with methotrexate-a known anticancer drug. Extracts of S.nigrum was found to be extremely effective in the prevention of cell proliferation of the mammary gland breast adenocarcinoma cell lines.* ***Discussion:*** *The pronounced antimitotic and anticancer activities of S.nigrum was due to its potential antioxidant property especially by the key role of phytochemicals such as polyphenols, steroidal saponin glycoside, alkaloids and flavonoids. Active principle sterol has been separated by TLC.* ***Conclusion:*** *These findings suggest that the promising antioxidant properties of the plant could be exploited in herbal preparations against oxidative stress, ageing, Ischemic heart disease in dissolving thrombus, microbial infections, hormone replacement therapy (HRT) and cancer justifying their use in traditional medicine.*

Keywords: *Solanum nigrum; Allium cepa;* Mammary carcinoma fibroblast-MCF-7 cell lines; Antimitotic; Anticancer.

INTRODUCTION:

The "functional food" industry has produced and marketed foods enriched with bioactive compounds, but there are no universally accepted criteria for judging efficacy of the compounds or enriched foods. The lack of understanding bioactive compounds and their health benefits should not serve to reduce research interest but should instead encourage plant and nutritional scientists to work together to develop strategies for improvement of health through food[1].

Cancer is the uncontrolled growth of cells coupled with malignant behavior: invasion and metastasis. Cancer is thought to be caused by the interaction between genetic susceptibility and environmental toxins. In the broad sense, most

ISSN (online): 2250-141X
Vol. 1 Issue 2, December 2011

chemotherapeutic drugs work by impairing mitosis (cell division), effectively targeting fast-dividing cells. As these drugs cause damage to cells they are termed cytotoxic. Some drugs cause cells to undergo apoptosis called "programmed cell death". The use of minerals and plant-based medicines are believed to date back to prehistoric medicine. In present work an attempt has been made to study the pharmacognosy of the traditional or tribal medicine i.e. *Solanum nigrum, leaves* of the plant are claimed to possess anti-tumor activity[2] and hence, efforts has been taken to determine the anticancer activity.

Another possible mechanism of action reported for anticancer drugs is inhibition of DNA synthesis and thus prevention of cell division. Folic acid supplied from the diet is essential for the production of terahydrofolic acid (THF). The conversion of folic acid to THF is carried out by an enzyme folate reductase. Anticancer drugs compete with folic acid for this enzyme thus restricting the production of THF required for synthesis of DNA and consequently for cell replication. Cells which do not have adequate production of THF eventually die[3,4].

Plants have been used in traditional medicine for several thousand years[5]. The knowledge of medicinal plants has been accumulated in the course of many centuries based on different medicinal systems such as Ayurveda, Unani and Siddha. In India, it is reported that traditional healers use 2500 plant species and 100 species of plants serve as regular sources of medicine[6]. During the last few decades there has been an increasing interest in the study of medicinal plants and their traditional use in different parts of the world[7-11]. Documenting the indigenous knowledge through ethnobotanical studies is important for the conservation and utilization of biological resources.

Today according to the World Health Organization (WHO), as many as 80% of the world's people depend on traditional medicine for their primary healthcare needs. There are considerable economic benefits in the development of indigenous medicines and in the use of medicinal plants for the treatment of various diseases[12].

Solanum nigrum Linn. (Family Solanaceae) is commonly used in the traditional medicine as a remedy for treating various diseases. The berries possess various medicinal properties such as sedative, diaphoretic, diuretic, hydragogue, expectorant and are useful in the disease of liver, heart and eyes and is also effective against piles, fever and dysentery[13]. The leaves are used to heal open wounds and are known to possess hypotensive effect[14]. The berries has been used in the treatment of stomach ulcers in the folk medicine in South Africa, European, China and through out India[15]. The fruits of *S. nigrum* have been reported to play an adjuvant role in the hepatoprotective property. Inhibition of lipid peroxidation and free radical scavenging activity has been suggested as a possible mechanism of action[16]. Previous studies have demonstrated that aerial parts of *S.nigrum* have the ability to decrease the secretion of gastric acid, pepsin level and stimulate mucus secretion[17].

The antimitotic activity was screened using *Allium cepa* root meristamatic cells which have been used extensively in screening of drugs with antimitotic activity[18,19]. The roots of all plants have distinguished regions, one of them being the region of cell division that lies beyond the root cap and extends a few mm after that. Cells of this region undergo repeated divisions. The fate of cell division is higher in this region compared to that of the other tissues. This region is called the meristamatic region (meristos: divided)[20]. This division is similar to the above mentioned cancer division in humans. Hence, these meristamatic cells can be used for preliminary screening of drugs with anticancer activity. Even though doubts can be raised about extrapolation of

ISSN (online): 2250-141X
Vol. 1 Issue 2, December 2011

results from plant tissue to animals and finally to humans, Khilman has noted that plant cells are 1000 times more resistant to colchicines which is a potent anti-carcinogen and which acts by inhibiting the microtubule formation. Thus, it is possible that chemicals that affect plant chromosomes will also affect animals[21].

S.nigrum extract is effective against *A.cepa* root cells; it will also have antimitotic effect against animal and human cells. To evaluate this hypothesis, it was thought worthwhile to evaluate the activity of the extract of S.nigrum on MCF-7-Human mammary gland breast adenocarcinoma cell lines. Phytochemical evaluation plays an important role in the standardization of crude herbal drugs[22,23].

MATERIALS AND METHODS:

Collection and processing of plant material: The plant *Solanum nigrum* was collected in Chennai, Tamil Nadu, India. The identification and nomenclature of the plant was based on The Flora of Presidency of Madras [24]and The Flora of Tamil Nadu Carnatic [25]. They were later verified at Botanical Survey of India, Southern Circle, Coimbatore, India. All the preserved specimens were deposited at the Herbarium of Entomology Research Institute, Loyola College, Chennai. The freshly collected leaves were washed and air dried in the shade at room temperature. Dried leaves and dried leafs were taken separately and powered for extraction. Fresh leaf was homogenized with solvent and then extracted. *Allium cepa* bulbs (red variety) were purchased from the local market and stored for the entire study.

Chemicals: Carmine stain and solvents were procured from Sigma Aldrich, New Delhi. India. Eagles minimal essential medium for cell proliferation, MTT, RPMI-1640 were purchased from LGC Promochem India Pvt. Ltd. Bangalore, India.

Preparation of plant extracts: The plant material (leaves) was air dried in the laboratory at room temperature. It was then powdered and extracted with hot water by boiling for 30 minutes to get the aqueous extract. The extract obtained was concentrated and dried under controlled temperature (60^0C). The dried powder was successively extracted with other solvents. Aqueous and Organic extracts were prepared. The dried powder was successively extracted with 70% ethanol, then with chloroform. Finally it was concentrated and made up to particular volume. Extraction with each solvent was done in a water bath for 60min with a reflux condenser. Each time before extracting with the next solvent the marc was dried in an air oven below 50^oC. Each extract was concentrated and evaporated to dry extract. Extracts of desired concentrations were prepared for further study using these dried extracts.

Plate: 1

Antimitotic activity: This activity was evaluated using *A.cepa* root meristamatic cells. *A cepa* were sprouted in tap water for 48 hr at room temperature. The bulbs that developed uniform root were used for

ISSN (online): 2250-141X
Vol. 1 Issue 2, December 2011

the experiment. These roots were treated with above prepared extracts of 10 concentrations. Water was used as medium/vehicle dilution. The different fractions used have been mentioned in Table 1. A blank with water was used as control. Methotrexate was used as a standard control. After 3hr of treatment, the root tips were fixed with fixing solution of acetic acid and alcohol. Squash preparations were made by staining the treated roots with acetocarmine stain. The mitotic index was calculated as

Mitotic Index = Number of dividing cells/Total number of cells x 100.

The aqueous and organic extracts were also subjected to preliminary phytochemical characterization, which revealed the presence of the phytochemicals-alkaloids, phenols, flavonoids, sterol, saponin glycosides, reducing sugars, proteins, cardio active aglycones and cardinolides, saponin glycosides. Folic acid added to the solution of methotrexate, aqueous extract and organic extract of *S.nigrum*. A similar experiment was undertaken to find out the probable mechanism of action through which the extracts and methotrexate act. Squash preparations made as above from the treated roots were observed.

Cell proliferation assay:

MCF-7- Human mammary gland breast adenocarcinoma cell lines were obtained from the American type culture collection and grown in the Minimal essential medium Eagles with L-glutamine and Earle's Basal salt solution adjusted to contain 1.5 g/liter sodium bicarbonate, 0.1.mM non-essential amino acids and 1 mM sodium pyruvate and supplemented with 0.01 mg/ml calf insulin - 90% and 10% fetal calf serum in a humidified atmosphere of 5 % co_2 at 37^{o}C.

The effect of *S. nigrum* on cell viability and growth was determined using MTT (3-4.5-dimethylthiazol-2-yl]-2, 5-diphenyl tetrazolium bromide) colorimetric assay [using a commercially available kit]. The compounds were dissolved in water as stock solution (1000×) and then diluted with RPMI-1640 for cell culture experiments. All solutions were prepared fresh on the day of testing. Human mammary gland breast adenocarcinoma cells were seeded at a density of 1×10^{4} per well in a 96 well plate.

After 24 hr, fresh medium was added containing aqueous extract of *S.nigrum* at concentration of

0, 10^{-4}, 10^{-3}, 10^{-2}, 10^{-1} and 10 mg/ml. After 4 days of incubation, in medium containing extract of S.nigrum cells were further incubated for 4 hr with the metabolic substrate, tetrazolium to formazan which was detected spectrophotometrically at 540 nm with multiwell spectrophotometer (ELISA Reader, Biotek Instruments Inc., Burlington,VT).

Preparative TLC: Slurry of silica gel GF_{254} was made in distilled water. This slurry was then applied on glass plates (12.5 x 12.5cm) with the aid of a TLC spreader to obtain preparative silica gel plates having thickness of about 0.5mm. The plates were dried in an oven at 105^{o}C and activated 2hr before use.

Sample preparation and application: Aqueous extract (1mg/ml) was spotted on the plate. The plates were developed using in solvent system. The plates were visualized and the spots were marked. Then the results were compared with standard sterol.

ISSN (online): 2250-141X
Vol. 1 Issue 2, December 2011

Plate 2

Statistical analysis: The data was subjected to statistic analysis using analysis of variance followed by appropriate post-hoc tests. . $P<0.05$ was considered as significant.

RESULTS:

Effect of aqueous extract of *S.nigrum* on mitotic activity: Antimitotic activity of aqueous extract was comparable to the activity of methotrexate (Table 1). The activity of organic extract was less than that of the aqueous extract. A one way ANOVA showed that there was a significant effect of treatment on mitotic activity. Post-hoc analysis using the Newman-Keuls test showed that the activity of all the different extracts were significant when compared with water (control). The aqueous extract showed lowest mitotic index i.e. highest activity amongst all the different extracts.

Table 1

Table 1 shows the antimitotic activity of different extracts and methotrexate. It was observed that the extract decreases the mitosis of *A.cepa* root tips. The phase was differentiated in each case and it was observed that the number of non-dividing cells increase with an aqueous extract than organic extract. A one-way ANOVA showed that there was a significant decrease in the mitotic index by aqueous and organic extracts of *S.nigrum* ($F=1266.2$; $P<0.001$) Post-hoc analysis using Newman-Keuls test showed that the aqueous extract was the most effective and it showed more or less similar effect to that of standard methotrexate-anticancer drug, which was not significantly different from the referred drug ($q=0.7016$; $P>0.05$). The cell divisions were differentiated and number of cells in each phases of cell division i.e. either prophase, metaphase, anaphase, or telophase were recorded. Thus, the number of cells entering prophase decreased. Since the cells do not enter prophase, further stages of cell division also decrease. Newman-Keuls test showed that the aqueous extract was the most effective. It was observed that the aqueous fraction showed better activity than organic fraction.

Effect of folic acid on antimitotic activity of *S.nigrum* and methotrexate: Analysis of data using a 3-way ANOVA showed that there was a significant effect of the pretreatment with folic acid on the antimitotic activity of *S.nigrum* and methotrexate.($F=144.65$). The mitotic index increased when folic acid was added to the aqueous, organic extracts of *S.nigrum* and methotrexate solution which otherwise reduce the mitotic activity in the absence of folic acid. This however, did not increase with increase in folic acid concentration suggesting that the effect was not dose-dependent. By comparing the mitotic index of methotrexate and *S.nigrum, it* was observed that incorporation of folic acid increased the mitotic index significantly in case of methotrexate, but not so in case of *S.nigrum.* Post-hoc analysis of the data showed that folic acid inhibited the anti-mitotic activity of methotrexate to a greater extent as compared to *S.nigrum.*

ISSN (online): 2250-141X
Vol. 1 Issue 2, December 2011

Table 2

Plate3 to Plates 13

DISCUSSION:

The result from the study showed that the aqueous extract of *S.nigrum* had excellent anti-mitotic activity that was comparable to the activity of methotrexate. Maximum numbers of non-dividing cells were observed. Methotrexate-anticancer drug competitively inhibits dihydrofolate reductase (DHFR), an enzyme that participates in the tetrahydrofolatesynthesis. Methotrexate acts specifically during DNA and RNA synthesis, and thus it is cytotoxic during the S-phase of the cell cycle. Logically, it therefore has a greater toxic effect on rapidly dividing cells such as malignant and myeloid cells[26,27]. The addition of folic acid inhibited the antimitotic activity of *S.nigrum* significantly[3], but does not completely inhibit the activity of methotrexate.

Folic acid is essential for the production of tertrahydrofolic acid (THF) which is in turn, required for synthesis of DNA and consequently for cell replication. Hence the dividing cells were high compared to that of the methotrexate treated cell.[28]

Aqueous extract of *S.nigrum* was also effective in reducing the cell viability of MCF-7-Human mammary gland breast adenocarcinoma cell lines that may be acting following the same mechanisms as those in the *Allium cepa* meristamatic cells. By virtue of this, if the extract is administered in humans it may prevent cell proliferation by directly combining with cell receptors/ enzymes and eliciting signals or cell apoptosis.

Phytochemical characterization of the different extracts revealed the presence of the phytochemicals- Indole alkaloids, polyphenols, flavonoids, sterol, saponin glycosides, reducing sugars, proteins, cardio active aglycones and cardinolides. Aqueous fraction contains steroids along with other polar constituents. Though, the probability of steroids extracted in a polar solvent was low, these steroids occurred as aglycones of the saponin glycosides after the glycosides hydrolyzed. There might be other polar compounds in the total extracts that might be acting synergistically with steroids of *S.nigrum.* The most common phytosterol are B-sitosterol, campesterol and stigmasterol[29]. *S.nigrum* is having these phytosterol. An important group of antioxidant, not previously mentioned, includes the sterol[30]. Phytosterol may offer protection from chemically induced colon cancer.[31]

Phytosterol has effect on apoptosis. The rate of tumor growth is dependant upon a balance between the rates of cell proliferation and apoptosis. Apoptosis is a programmed cell death, as influenced by phytosterol.[32,33] Hence, the sterols from *S.nigrum* must be contributing to the anticancer potential of the herb.

The aqueous extract of *S.nigrum* seems to prevent prophase stage in cell division where DNA duplication occurs. Methotrexate is a known anticancer drug that inhibits DNA synthesis. When folic acid was supplemented to the methotrexate and the total aqueous extract, it was seen that mitotic index increased. Thus, it may be suggested that the extract may be acting through the pathway inhibiting tetrahydrofolic acid and hence folic acid is required for DNA synthesis that arrest cell division. Methotrexate is known as

ISSN (online): 2250-141X
Vol. 1 Issue 2, December 2011

anticancer drug which compete with folic acid for the enzyme reductase[25,26]. The total aqueous extract of *S.nigrum* may also be competing with folic acid thus inhibit the DNA synthesis. Hence, addition of folic acid increases the mitotic index due to the availability of folic acid. However, the mitotic index does not increase significantly in case of *S.nigrum* as compared to that of methotrexate. This may be because the extract may be mediating its effects through other mechanisms also. The extract binding with different cell proteins are responsible for cell division. This effect may be due to steroidal glycosidic alkaloid or steroidal alcohol.[32,33]

The finding of this study indicate that *S.nigrum* is a promising source of steroidal glycosidic alkaloid or steroidal alcohol, polyphenols, flavonoids, iodole alkaloid and FRSA, which have the ability to modify the physiological function of cells and hence act as anti-cancer drugs to arrest the proliferation of cancer cells. The extract shows commendable antioxidant activity which also may be one of the contributing factors to its anticancer potential. In the present study extracts of *S.nigrum* was tested for the antitumor activity and it showed most effective inhibition of MCF-7- Human mammary gland breast adenocarcinoma cell proliferation. Apoptosis is a critical molecular target by dietary bioactive agents for the prevention of cancer. The potential use of *S.nigrum* as therapeutic agent holds great promise as the isolation of one or more cytotoxic chemicals from crude extract and the judicious use of such chemicals can control the progression of cancer and also can prevent the formation of tumour in individuals who are highly susceptible to developing a tumour.

REFERENCES:

1. Finley JW. Reduction of cancer risk by consumption of selenium-enriched plants: enrichment of broccoli with selenium increases the anticarcinogenic properties of broccoli. J Med Food 2003; 6(1):19-26.
2. Naem Qaisar Bashir Ahmad Chaudhary, Altaf Dasti. Phytochemical study of aerial parts of Lantana camara for the pharmacological active compounds. J App Pharm 2009; 1(1):19-26.
3. Satoskar R S, Bhandarkar S D. Pharmacology and pharmacotherapeutics, 2nd edition.Bombay,Popular Prakashnan Publishers,1989;704.
4. Lehninger L A, Nelson. Principles of biochemistry, 1st edition.Delhi, CBS Publishers and Distributors, 1993; 725.
5. Abu-Rabia A. Urinary diseases and ethno botany among pastoral nomads in the Middle East: Journal of Ethnobiology and Ethnomedicine[Internet].2005 Aug[cited 2005 Aug 2];4(1):[about 1p.].Available from:http://www.ethnobiomed.com/content/1/14
6. Pei SJ. Ethno botanical approaches of traditional medicine studies: Some experiences from Asia.Pharmaceutical Biology 2001; 39:74-9.
7. Lev E. Ethno-diversity within current ethno-pharmacology as part of Israeli traditional medicine – A review [Internet].2005 Nov [cited 2006 Jan 9];2(1):[about 1p.].Available from:http://www.ethnobiomed.com/content/2/1/4
8. Gazzaneo LR, Paiva de Lucena RF, Paulino de Albuquerque U: Knowledge and use of medicinal plants by local specialists in an region of Atlantic Forest in the state of Pernambuco [Internet].2005 Sep [cited 2005 Nov 1]; 9(1): [about 1 p.]. Available from http://http.//www.ethnobiomed.com/content/1/1/9
9. Al-Qura'n S. Ethnobotanical survey of folk toxic

ISSN (online): 2250-141X
Vol. 1 Issue 2, December 2011

plants in southern part of Jordan. Toxicon 2005; 46:119-26.
10. Hanazaki N, Tamashiro JY, Leitao-Filho H, Gegossi A. Diversity of plant uses in two Caicaras communities from the Atlantic forest coast, Brazil. Biodiversity and Conservation 2000; 9:597-615.
11. Rossato SC, Leitao-Filho H, Gegossi A. Ethnobotany of Caicaras of the Atlantic forest coast (Brazil).Economic botany 1999; 53:387-95.
12. Asima, Chatterjee, Satyesh, Chandra. The Treatise on Indian Medicinal Plants,1st edition.Delhi,
Pakrashi Publications and Information directorate,1995;358-70.
13. Rastogi and Mehrotra. Compendium of Medicinal Plants. Pakistan Council of Science and
Industrial Research, Peshawar 1991; 134-5.
14. Ye,J.R. The hypotensive effect of Solanum nigrum. Zhong Yao Tong Boa 1984;9:35-36.
15. Ikram M, Hussain S.F. Compendium of Medicinal Plants.Pakistan Council of Science and
Industrial Research, Peshawar 1918; 134-5.
16. Sarwat Sultana,Shahid Pervaiz, Mohammed Iqbal. Crude extract of hepatoprotective plants,Solanum nigrum and Cichorum intybus inhibit free radical mediated DNA damage. J.Ethnopharm 1995; 45: 189-92.
17. Akthar M.S, Munir M. Evaluation of antiulcerogenic effect of S.nigrum, Brassica oleracea and Ocimim basilicum in rats. J. Ethnopharm 1989; 27: 163-76.
18. Abhang R Y. Jogiekar P P, Kulkarni P H. Preliminary study on the effect of T.cordifolia on mitosis. Ancient Sci 1991; 1:27.
19. Latha P G, Chandralekha C T, Vilasini G, Panikkar K R. Effects of the flower extract of lxora coccines linn. On the meristametic cells of A.cepa, Ancient Science of Life 1998; 17:42-62.
20. Dutta A C. A text book of botany, 14th edition.Delhi, Oxford University Press, 1971; 29.
21. Williams G O, O moh L E. Mitotic effects of aqueous leaf extract of Cymbopogo citrates in Allium cepa root tips. Cytobios 1996; 87: 161.
22. Aggarwal A. Standardisation of herbal drugs. Express Pharma Pulse 2001; 7:4621.
23. Spreeman R, Gaedeke T. Herbal grugs. Express Pharma Pulse 2001; 7:4621.
24. Gamble JS. The Flora of the Presidency of Madras. 1st edition. London, Adlard and son LTD, 1935; 460.
25. Matthew KM. The Flora of the Tamil Nadu Carnatic. The Rapinat Herbarium, St Joseph's College, Tiruchirapalli, India 1983.
26. Rajagopalan, P. T. Ravi, Zhang, Zhiquan; McCourt, Lynn et al. Interaction of dihydrofolate reductase with methotrexate: Ensemble and single-molecule kinetics. Proceedings of the National Academy of Sciences 2002; 99:21.
27. Johnston, Andrew, Gudjonsson, Johann Eli, Sigmundsdottir, Hekla et al. The anti-inflammatory action of methotrexate is not mediated by lymphocyte apoptosis, but by the suppression of activation and adhesion molecules. Clinical Immunology 2005; 114 (2): 154–63.
28. Satoskar R S, Bhandarkar S D. Pharmacology and pharmacotherapeutics, Vol.2, 11th edition. Mumbai, Popular Prakashnan Publishers, 1989; 704.
29. Vahouny G.V. Connor W.E., Subramanian S., Lin, D.S, Galio, L.L. Comparative lymphatic absorption of sitosterol, stigmasterol and frucosterol, and differential inhibition of cholestrol absorption. Am.J.Clin.Nutr 1983; 37:805-9.
30. Mdhavi D L, Deshpande S S. Food Antioxidants, In, Salunkhe D K (ed), Marcel

ISSN (online): 2250-141X
Vol. 1 Issue 2, December 2011

Dekker, 1st edition. New York, 1996; 4-64.
31. Raicht, R.F., Cohen, L. i., Fazzini, E.P., Sarwal, A.N, Takahashi, M. Protective effect of planet sterols against chemically induced colon tumors in rats. Cancer Res 1980; 40:403-5.
32. Awad A.B.,Downie D, Fink, C.S Inhibition of growth and stimulation of apoptosis by B-sitosterol treatment of breast cancer MDA-MB-231 cells in culture. Int. J.Mol.Med. 2000; 5:541-5.
33. Awad A.B, Gan y, Fink, C.S. Effect of B-sitosterol, a plant sterol on growth, protein phosphatase 2A and phospholipid D in LNCaP cells. Nutr Cancer 2000; 36:74-8.

Sl.No	Different solutions used for treatment	% of Non-dividing cells	% of dividing cells				Mitotic Index	Mitotic Index		
			P	M	A	T		Avg	SD	SEM
1	Water(control)	14	78	5	2	1	86	86.2	0.8367	0.3742
2	Methotrexate (Standard)	57	38	3	1	1	43	43	1.000	0.4472
3	Aqueous extract	57	39	2	1	1	43	43.4	1.817	0.8124
4	Organic Extract	44	50	2	3	1	56	56	1.225	0.5477

Table 1: Antimitotic activity after treatment of *A.cepa roots* with aqueous, organic extracts *of Solanum nigrum* and methotrexates

P-Prophase, M-Metaphase, A- Anaphase, T-Telophase

ISSN (online): 2250-141X
Vol. 1 Issue 2, December 2011

Sl.No	Different solutions used for treatment	% of Non-dividing cells	% of dividing cells				Mitotic Index	Mitotic Index		
			P	M	A	T		Avg	SD	SEM
1	Water(control)	14	78	5	2	1	86	86.2	0.8367	0.3742
2	Methotrexate (Standard)	57	38	3	1	1	43	43	1.000	0.4472
3	Aqueous extract	57	39	2	1	1	43	43.4	1.817	0.8124
4	Organic Extract	44	50	2	3	1	56	56	1.225	0.5477

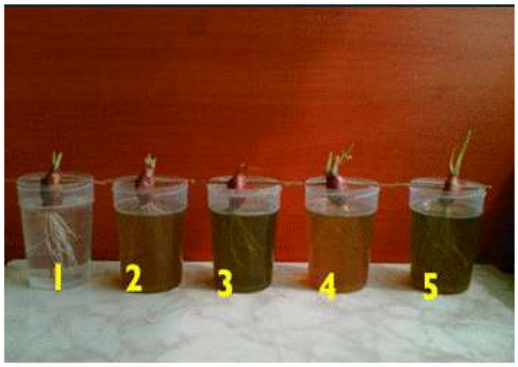
Table 2: Antimitotic activity after treatment of *A.cepa* roots with aqueous extract of *S.nigrum*+ folic acid, organic extract of *S.nigrum* + folic acid and methotrexate + folic acid

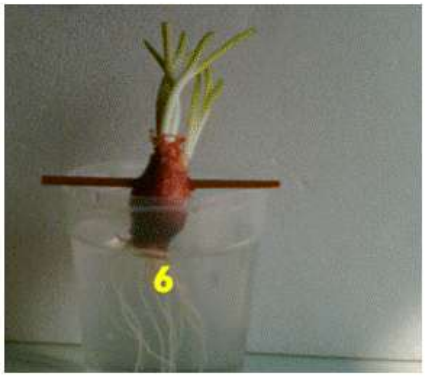
Sl.No	Different solutions used for treatment	% of Non-dividing cells	% of dividing cells				Mitotic Index	Mitotic Index		
			P	M	A	T		Avg	SD	SEM
1	Methotrexate + Folic acid	22	67	5	3	3	78	77.8	0.8367	0.3742
2	Aqueous + folic acid	33	63	1	2	1	67	66.6	1.517	0.6782
3	Organic + folic acid	24	68	3	3	2	76	75.8	0.8367	0.3742

P-Prophase, M-Metaphase, A- Anaphase, T-Telophase

ISSN (online): 2250-141X
Vol. 1 Issue 2, December 2011

Plate: 1 Treatment of Allium cepa roots with different extracts of Solanum nigrum and solutions

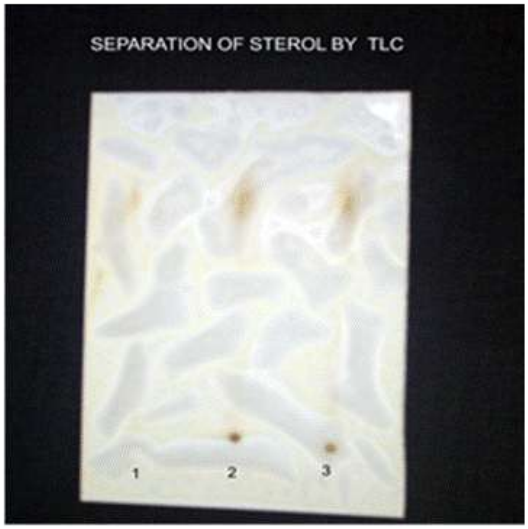




1. Standard – Methotrexate, 2. Aqueous extract, 3. Organic extract,
4. Aqueous + folic acid, 5. Organic + folic acid, 6. Water

ISSN (online): 2250-141X
Vol. 1 Issue 2, December 2011

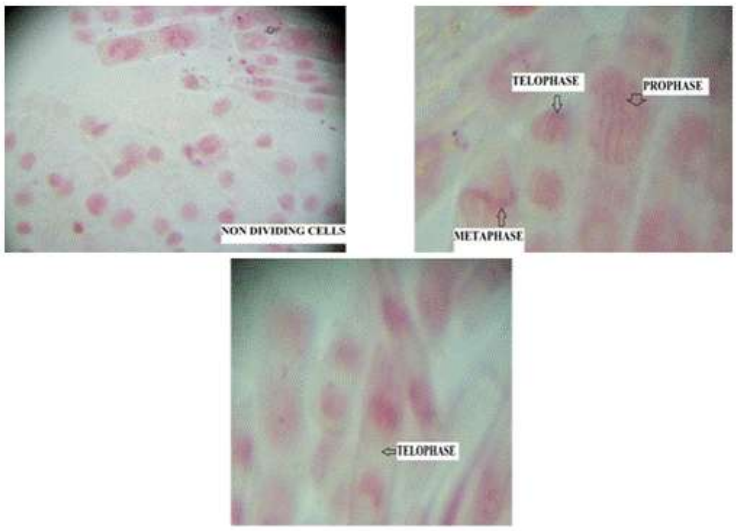
Plate 2: Separation of Sterol by TLC



1. Standard sterol
2. Aqueous extract of Solanum nigrum
3. Organic extract of Solanum nigrum

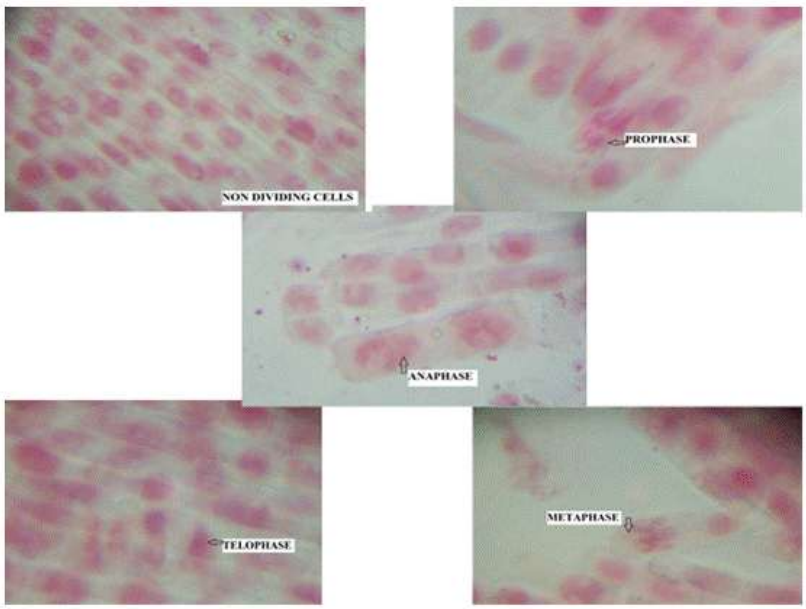
ISSN (online): 2250-141X
Vol. 1 Issue 2, December 2011

Plate3: Different stages of mitosis of Aillum cepa roots after treatment with water



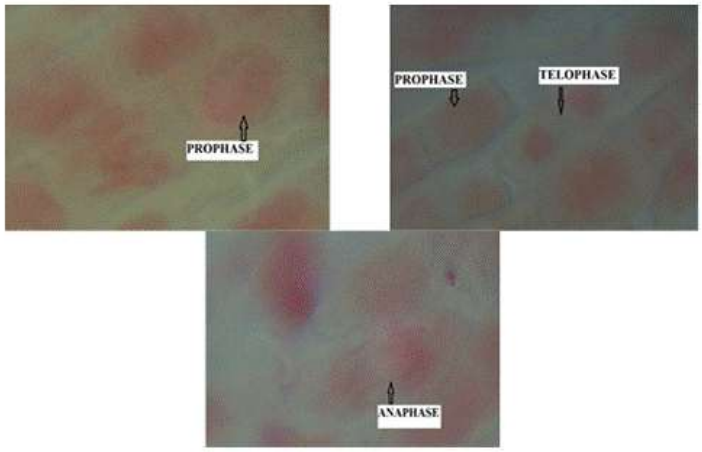
ISSN (online): 2250-141X
Vol. 1 Issue 2, December 2011

Plate-4: Antimitotic activity of aqueous extract of S.nigrum and the stages of cell division



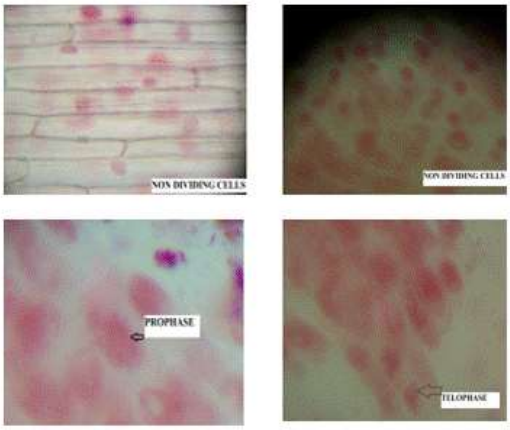
ISSN (online): 2250-141X
Vol. 1 Issue 2, December 2011

Plate-5: Antimitotic activity of organic extract of S.nigrum and the stages of cell division



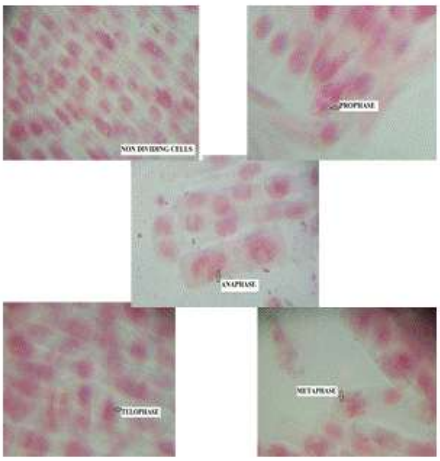
ISSN (online): 2250-141X
Vol. 1 Issue 2, December 2011

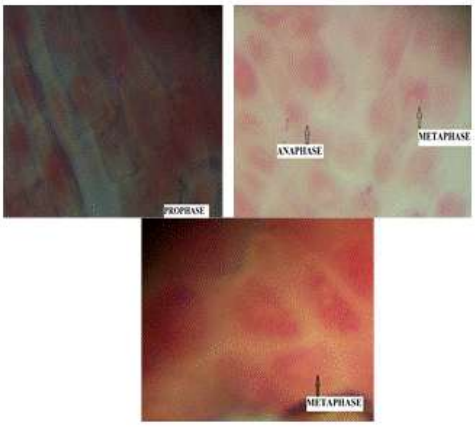
Plate-6: Antimitotic acivity of methotrexate, methotrexate + folic acid and the stages of cell division



ISSN (online): 2250-141X
Vol. 1 Issue 2, December 2011

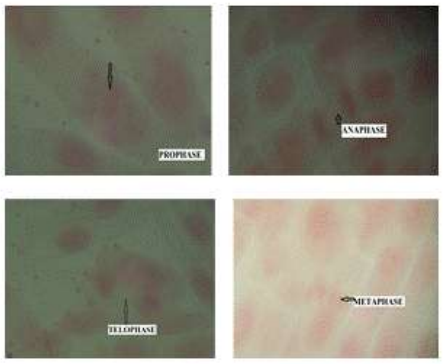
Plate 7: Antimitotic activity of aqueous leaf, aqueous leaf + folic acid extracts of S.nigrum and the stages of cell division – Plate a and Plate b

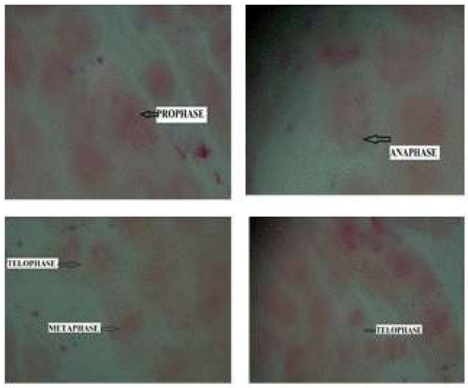




ISSN (online): 2250-141X
Vol. 1 Issue 2, December 2011

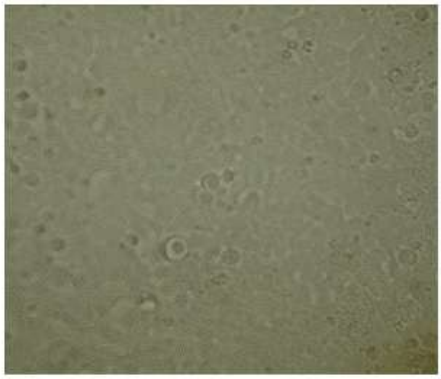
Plate 8: Antimitotic activity of organic leaf, organic leaf+ folic acid extracts of *S.nigrum* and the stages of cell division
(Plate a and Plate b)





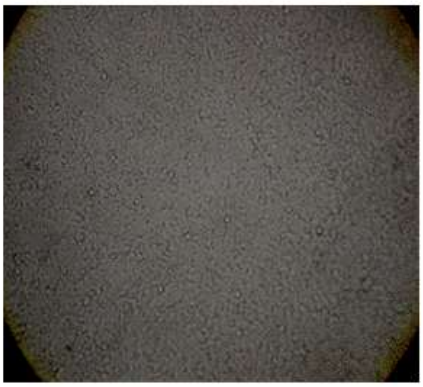
ISSN (online): 2250-141X
Vol. 1 Issue 2, December 2011

Plate-9: Normal MCF-7 Human mammary gland breast adenocarcinoma cell lines –showing confluent monolayer (20X magnification)



ISSN (online): 2250-141X
Vol. 1 Issue 2, December 2011

Plate-10: Anticancerous effect of aqueous dry leaf extract of S.nigrum on MCF-7 cell lines 12 hrs post infection showing cell rounding (40X magnification)



ISSN (online): 2250-141X
Vol. 1 Issue 2, December 2011

Plate-11: Anticancerous effect of aqueous dry leaf extract of S.nigrum on MCF-7 cell lines -24 hrs post infection showing cell rounding (40X magnification)

ISSN (online): 2250-141X
Vol. 1 Issue 2, December 2011

Plate-12: Anticancerous effect of aqueous dry leaf extract of S.nigrum on MCF-7 cell lines -32 hrs post infection showing cell growth inhibition and prevention (40X magnification)

ISSN (online): 2250-141X
Vol. 1 Issue 2, December 2011

Plate-13: Effects of aqueous dry leaf extract of S.nigrum on MCF-7 cell lines -38hrs post infection complete cell death and depopulation (40X magnification)