Inhibitory property of chloroform and ethyle extract of Solanum nigrum L. whole plant on proliferation of melanoma treated against A375 cell lines

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Abstract
Medicinal plants besides therapeutic agents are also a big source of information for a wide variety of chemical constituents which could be developed as drugs with precise selectivity. This study was intended to evaluate the anti-cancerous activity of chloroform and ethyle acetate extract of Solanum nigrum L. whole plant on the human melanoma cancer cell lines (A375). The percentage viability of cancer cell lines was carried out by using trypan blue dye exclusion method and cytotoxicity assay was screened by applying MTT [3-(4, 5-Dimethylthiazol-2-yl)-2, 5-Diphenyl tetrazolium Bromide] assay. Solanum nigrum L. chloroform extract has depicted momentous and potent cytotoxic effect on A375 cell lines with IC₅₀ 346µg/ml in concentration ranging between 50µg/ml to 750µg/ml and acted as best agent to reduce the number of cancer cells to minimum. Further studies are needed to better characterize the mechanism of action underlying the beneficial effects of this alkaloid on their pathologies. Hence Solanum nigrum L. can be considered to be important to further investigate on molecular level to ascertain mechanism of action for said activity.

Keywords: Solanum nigrum L., anticancerous activity, A375 cell line, MTT assay Trypan blue dye

Introduction
Solanum nigrum L. (Kamhali) (Kashmiri) has been traditionally used to treat pathological ailments like fever, ulcers, bacterial infections, fungal infections, jaundice and liver disorders (Creasy et al., 1981; Capizzi et al., 2003; Sudhanshu et al., 2000 and Borgia et al., 1981) [2]. The most important constituents from Solanum nigrum (i.e., vincristine, irinotecan, camptothecines) and microorganisms (i.e., doxorubicin, daclactinomines, mitomycin and bleomycin) (Grever, 2001) [6]. The history of Solanum nigrum L. dates back to ancient China and the Mediterranean region as a highly popular laxative drug and a general tonic (Dashputre et al., 2010) [3]. It is used as purgative and astringent tonic; its stimulating effect combined with apparent properties renders it especially useful in tonic dyspepsia (Chintana et al., 2012) [4]. Powdered roots are sprinkled over ulcer for healing. Leaf and berries are eaten either raw or boiled, sprinkled with salt and pepper. Some workers have worked out antitumor activity of Solanum nigrum L. (Anindyajati et al., 2010) [1] but very little is known about the mechanisms involved. The endeavor of the present study was to travel around the potential anti-cancerous activity of Solanum nigrum L. whole plant on melanoma cell lines. The study was not only supportive in determining the optimum dose of solanine toxin employed against melanoma cancer but also in the development of a new and a potential anti-cancer drug. The aqueous chloroform extract of Solanum nigrum L. whole plant is an effective and potent anti-cancerous agent against human melanoma cancer cell lines so it may provide a poor man friendly and a drug of preference to the world.

Materials and methods
Methods of Preparation of Plant Extract
The plant Solanum nigrum L. was collected and washed thoroughly under running tap water and then rinsed in distilled water and allowed to dry for some time. Then the plant was shade dried without any contamination for about 3 to 4 weeks. The powder was extracted according to (Rashmi et al., 2010).
The dried plant was powdered (coarse) and subjected to Soxhlet apparatus using ethyl acetate and chloroform respectively. Almost all the chlorophyll and lipid is deposited on the side of the flask and was removed carefully. The extraction was done with each solvent until the supernatant in the Soxhlet became transparent for 36 hours. Every time before taking the solvents of higher polarity to remove the traces of previous solvents, exhausted marc was completely dried. All the extracts were filtered, dried and weighed.

Cell counting was performed by applying trypan blue dye: Calculated the number of cells per ml, and the total number of cells, using the following formula:-
% Viability = (Live Cell Count/ Total Cell Count x100)

Micro culture tetrazolium (MTT) assay Procedure: The monolayer cell culture was trypsinized and the cell count was adjusted to 3-1 lakhs cells/ml using medium containing 10% newborn calf serum. To each well of 96 well microtitre plates, 0.1 ml of diluted cell suspension was added. After 24 hours, when the monolayer formed the supernatant was flicked off and 100 μl of different test compounds were added to the cells in microtitre plates and kept for incubation at 37°C in 5% CO₂ incubator for 72 hour and cells were periodically checked for granularity, shrinkage, swelling. After 72 hour, the sample solution in wells was flicked off and 50μl of MTT dye was added to each well. The plates were gently shaken and incubated for 4 hours at 37ºC in 5% CO₂ incubator. The supernatant was removed, 50 μl of Propanol was added, and the plates were gently shaken to solubilize the formed formazan. The absorbance was measured using a microplate reader at a wavelength of 490 nm. The percentage growth inhibition was calculated using the formula below: The percentage growth inhibition was calculated using following formula, % Cell Inhibition=100−{(At−Ab)/(Ac−Ab)}×100
Where, At= Absorbance value of test compound, Ab= Absorbance value of blank, Ac=Absorbance value of control

Percentage cell survival rate was calculated by applying formula, % Cell Survival = {((At−Ab)/(Ac−Ab))}×100, Where, At= Absorbance value of test compound, Ab= Absorbance value of blank, Ac = Absorbance value of control, % cell inhibition= 100-cell survival.

Result and Discussion
Viability and Characterization of Cell Lines
Cell lines derived from NCCS, Pune were free from any type of bacterial and fungal infectivity. In-vitro studies on ethyl acetate and chloroform extract of Solanum nigrum L. showed significant antitumor activity against human melanoma cell line (A 375). The viable cell percentage was obtained by performing trypan blue dye exclusion technique and cytotoxicity activity was conceded out by using by MTT assay.

To measure the cytotoxicity of chloroform and ethyl acetate extract of Solanum nigrum L. in human cell lines (A375). A375 cells were cultured with chloroform extract (0.01, 0.1, 1, 10, 100 μg/ml) and (0.01, 0.1, 1, 10, 100 μg/ml) with ethyl acetate extract or without extract for 12hrs to 96hrs. IC₅₀ value was experimentally calculated to be 55.93 mg/ml for chloroform and 64.67 mg/ml for ethyl acetate extracts. As chloroform extract having low IC₅₀ was subjected to further proceeding. Cell viability was evaluated by trypan blue dye exclusion method. By using trypan blue test, chloroform extract exhibited a remarkable reduction against A375 cell viability in a concentration dependent manner, these results are in concordant with MTT assay. Thus inhibition of cell growth by a chloroform extract was more pronounced at concentration of (100μg/ml). On comparing both the extracts, chloroform extract showed high cell viability. In vitro screening revealed that the aqueous extract of Solanum nigrum L. exhibits significant activity against A375 cell line. The Solanum nigrum L. extract showed potent activity against cell viability in a dose dependant manner (Graph 1). Cytotoxic upshot of aqueous extract of Solanum nigrum L. against melanoma cell line (A375), when it was being incubated for 72hrs with 3 (4, 5-dimethyl thiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) (Graph 2). IC₅₀ value was experimentally calculated to be 55.93 mg/ml for chloroform and 64.67 mg/ml for ethyl acetate extracts. The aqueous extract showed cytotoxicity in a concentration dependant manner. In the present study in-vitro screening of both extracts revealed that the percentage cell survival rate decreased significantly at 100μg/ml extract treatment. When the A375 melanoma cells were treated with 1µg/ml cisplatin alone, there was considerable reduce in the percentage of cell survival rate. But when the same cells were treated with 100µg/ml extract adjuvant with 1µg/ml cisplatin, there was remarkable turn down in the percentage of cell survival rate.

Similarly Lin et al., (2005) observed that Solanone is a newly isolated alkaloid which has a strong inhibitory effect on the growth of tumor cells. By using cell cultures, it was demonstrated that the tumor cells were more sensitive to alkaloid than non-transformed cells and that this could be caused by the higher binding affinity of alkaloid to tumor cells than to non-transformed cells. This revealed that both extract were having significant anticancer potential but amongst them chloroform extract was more potent.
Graph 2: Representing significant activity of chloroform extract of *Solanum nigrum* L. against A375 cell line.

The extract showed activity against cell viability in a dose dependant manner. There was considerable decline in cell viability at given extract concentration of 100µg/ml.

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References