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Original Article

Investigation of cytotoxicity induced by *Nigella sativa* and *Azadirachta indica* using MDA-MB-231, HCT 116 and SHSY5Y cell lines

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ABSTRACT

Background: Indian herbal plants are widely used as medicine in the ancient ayurvedic and culinary purposes. *Nigella sativa* that also called as in black cumin is a flowering plant in the family *ranunculiceae* that is native to Southeast Asia. More recently *Nigella sativa* is also been used as anti cancer drug and protective agent against gamma radiation induced adverse effects in cell lines. *Azadirachta indica* commonly called as neem, is a tree belonging to mahogany family meliaceae. *Azadirachta indica* is also a traditional medicinal plant that used from a very long time in Indian ayurvedic and it is also been reported to have many beneficial effects including but not limited to anti-cancer and anti-diabetic effects. **Objective:** Find the IC_{50} values of *Nigella sativa* and *Azadirachta indica* ethanolic extracts in MDA-MB-231, HCT 116 and SHSY5Y cell lines. **Methods:** In this study we selected two cancerous cell lines (MDA-MB-231, HCT 116) and one neuronal cell line (SHSY5Y) and studied the effect of the two plant extracts namely *Nigella sativa* and *Azadirachta indica* on the cell metabolic activity. **Results:** This study revealed that cancerous cell lines are more prone to the plant extracts than the neuronal cell lines. These results suggest positive clues on how such medicinal plant extracts act against cancerous cells alone while affecting the normal cells to a limited extent. However, further studies are required to find if this effect is due to cytotoxicity, cytostaticity, or anti-adhesive property.

Key words: Herbal plants, Ayurvedic medicine, Cancer cells, Neuronal cells.

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INTRODUCTION

In the recent times an exponential growth in the field of herbal medicine has been observed and these drugs are gaining popularity both in developing and developed countries because of their natural origin and reduced side effects.[1,2] Many traditional medicines in use are derived from medicinal plants, minerals and organic matter and a number of medicinal plants, termed rasayana are used in the Traditional Indian health care systems have been in use for over 1000 years. Among the 21,000 plants listed by The World Health Organization (WHO) which are used for medicinal purposes around the world, 2500 species are in India, out of which 150 species are used commercially on a fairly large scale. India is the largest producer of medicinal herbs and is very aptly known as the botanical garden of the world.[3]

Azadirachta indica commonly called as neem is being traditionally used from a very long time due to its various beneficial effects including anti-cancer and anti-diabetic effects.[4] More recently *Nigella sativa* commonly called as black cumin, is also being used in cancer treatment and is also a protective agent against gamma radiation-induced adverse effects in cell lines.[5,6,7] In order to understand the effects of the plants on the cell metabolic activity, the plant extracts were prepared and we selected two cancerous cell lines (MDA-MB-231- breast adenocarcinoma, HCT-116 – colon carcinoma) and one non-cancerous neuronal cell line (SHSY5Y). This approach is thus made in order to develop successful therapeutic modalities for anticancer activity. The development of such drugs will require a comprehensive understanding of the characteristics of cancer cells as well as applying modern technologies for drug delivery. This paper emphasis on medicinal plant based therapeutics to ascertain whether herbal extracts differentially cause cell death in cancer and normal cells.

METHODOLOGY

Materials Used

(3-[4,5-dimethylthiazol-2-yl]-2, 5-diphenyl tetrazolium bromide) or MTT, DMSO.95% ethanol. All chemicals and reagents used were of analytical quality.

Plant extracts preparation

The *Nigella sativa* seeds and *Azadirachta indicaleaves* were washed with water, shade dried and extracted by boiling 500 g of the seed powder (twice) in 3000 mL of 95% Ethanol for 30 min at 70°C in a soxhlet extraction unit. The extract obtained was flash evaporated and concentrated on a water bath at

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atmospheric pressure to a semisolid condition, which was further dried in an oven at 30°C on a shallow dish to remove the solvent completely.

Phytochemical screening

Preliminary phytochemical analysis of the extract was performed by simple chemical tests.

MTT Assay

The cells were seeded at 50,000 cells / well (HCT-116, SHSY5Y, MDAMB231) in a 96 well plate and incubated for 24 hrs at 37°C, 5 % CO_2 incubator. The samples to be tested were added from 0-320µg/ml (2 fold variation) concentration of DMEM without FBS was incubated for 24 hrs.After incubation the test samples were added with 100µl/well (50 mg /well) of the MTT (5 mg/10ml of MTT in 1X PBS) to the respective wells and incubated for 3to 4 hours (Figure 1).After incubation, the MTT reagent was discarded by pipetting without disturbing cells and 100 µl of DMSO was added to rapidly solubilize the formazan.The Absorbance was measured at 590 nm.[8, 9]

RESULTS AND DISCUSSION

Nigella sativa and *Azadirachta indica* extract samples were tested by MTT assay[11, 12] and the following results were obtained for three different cell lines HCT116, MDAMB231 and SHSY5Y. All the cell lines were tested with various concentrations of plant extracts 10, 20, 40, 80, 160 and 320 µg/ml. And a graph was drawn with extract concentration as x-axis and corresponding observance at 590 nm as y-axis. The IC_{50} value was calculated from drawing a tread line connecting all the points plotted and an equation for the line was generated. Using the equation IC_{50} value was obtained as 119.5, 125.8 and 224.7 µg/ml for HCT116, MDAMB231 and SHSY5Y cell lines in the case of *Nigella sativa*. And in the case of *Azadirachta indica* 50.52, 131.1 and 162.8 µg/ml for HCT116, MDAMB231 and SHSY5Y cell lines. The calculated IC_{50} values are mentioned in Table 1 and the detailed observance and corresponding % inhibition of growth for each cell line and samples are mentioned below in Table 2.

The half maximal inhibitory concentration (IC_{50}) is a measure of the effectiveness of a substance in inhibiting a specific biological or biochemical function.[13] This quantitative measure indicates how much of a

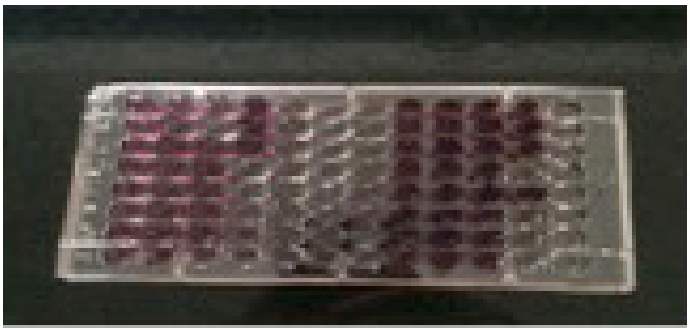

Figure 1: MTT assay microplate.

Table 1: IC_{50} values of *Nigella sativa* and *Azadirachta indica* in HCT116, MDAMB231 and SHSY5Y

Cell lines	IC_{50} µg/ml (*Nigella sativa*)	IC50 µg/ml (*Azadirachta indica*)
MDAMB231	125.8	131.1
HCT116	119.5	50.52
SHSY5Y	224.7	162.8

Table 2: MTT assay values, observance at 590 nm, % inhibition and IC_{50} values of extracts

Samples	Cell lines	Conc. µg/ml	OD 590 nm	% Inhibition
Nigella sativa	Control	0	0.657	0
	HTC116	10	0.626	4.71
		20	0.579	11.88
		40	0.521	20.59
		80	0.398	39.32
		160	0.327	50.19
		320	0.202	69.21
	Control	0	0.609	0.00
	MDA-MB-231	10	0.574	5.67
		20	0.519	14.66
		40	0.468	23.07
		80	0.371	39.03
		160	0.247	59.43
		320	0.156	74.30
	Control	0	0.308	0
	SHSY5Y	10	0.295	4.18
		20	0.265	14.11
		40	0.239	22.61
		80	0.206	33.21
		160	0.159	48.33
		320	0.086	72.14
Azadirachta indica	HTC116	10	0.617	6.01
		20	0.549	16.45
		40	0.406	38.18
		80	0.354	46.02
		160	0.264	59.74
		320	0.161	75.54
	MDA-MB-231	10	0.570	6.31
		20	0.498	18.10
		40	0.450	26.04
		80	0.346	43.15
		160	0.231	62.04
		320	0.115	81.08
	SHSY5Y	10	0.291	5.48
		20	0.270	12.39
		40	0.245	20.53
		80	0.207	32.79
		160	0.169	45.15
		320	0.112	63.67

particular drug or other substance (inhibitor) is needed to inhibit a given biological process (or component of a process, i.e. an enzyme, cell or microorganism by half. The results suggest that the plant extracts *Nigella sativa andAzadirachta indica* have effects on HCT-116, SHSY5Y and MDAMB231 cell metabolic activity compared to control and can be considered for further studies. *Nigella sativa and Azadirachta indica* extract has the advantages of easy availability, low cost and safety to humans, which collectively make plant derived compounds valuable candidates for anticancer therapy. Preclinical studies have primarily established neem as a potential preventive and therapeutic agent against various types of cancer. Many investigators now believe that traditional medicine is a promising source of new therapeutics against cancer. Extensive research with *Nigella sativa and Azadirachta indica* may contribute to the discovery of new anticancer strategies.

The %inhibition is calculated using the formula:

% Inhibition *= 100 – (OD of sample/OD of Control) × 100.*

CONCLUSION

This study revealed that cancerous cell lines are more prone to the effects of the plant extracts than the neuronal cell lines. These results suggest positive clues on how such medicinal plant extracts act against cancerous cells alone while affecting the normal cells to a limited extent.[14] Therefore, this preliminary study will lead to study the mechanism behind this effect.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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