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CYTOTOXICITY EFFECT OF *SYZYGIUM CUMINI* SEED AND *NIGELLASATIVA*SEEDEXTRACTS ON HUMAN HEPATO CELLULAR CARCINOMA

Education

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ABSTRACT

The purpose of this study was to assess the cytotoxicity effect of *SC* and *NS* in the human liver hepatocellular carcinoma, HepG2. The test items *SC* and *NS* has been tested in the MTT assay for the cell growth inhibition property in human hepatocellular carcinoma cell line, HepG2. The results revealed that the compounds does not showing any much toxicity to the tested cell line. However compound *Syzygium cumini* shows slightly higher toxicity than compound *Nigella sativa* at the higher concentration tested wells. Cells treated with *S. cumini* and *N. sativa* images shows no significant difference between the same concentrations of compound treated wells.To evaluate the test items (*S.cumini and N.sativa*) cytotoxic property using HepG2 cell line.*S.cumini* and *N.sativa* Concentration: 1000, 300, 100, 30, 10, 3.0, and 1.0 µg/ml. Cell line: HepG2 (Human Liver hepatocellular carcinoma).

KEYWORDS

S.cumini, N.sativa, Ethanol Extract, Cytotoxicity, HepG2, Carcinoma cell line.

INTRODUCTION

The plants have medicinal values and used to treat the cancer cells. Medicinal herbs have chemotherapeutic properties to killing capabilities in different types of human derived tumor cell lines. Plants have been used for medical purposes since the beginning of human history and are the basis of modern medicine. Cell death caused by some plant seed extracts is through apoptosis.). *Clinical Studies of Experience Herbal Composite Formula.* Because the focus of composing a formula is notin the direct "killing" of tumor cells, herbal composite formula is usually considered as palliative treatment and adjuvant treatment for HCC. The purpose for clinical use of experience herbal composite formula is to recover liver function, alleviate symptoms and to improve life quality.*Syzygium cumini* seed extracts strongly showed anticancer effects peculiarly on Hepatic carcinoma cells. Human hepato cellular carcinoma (HCC) constitutes about 85% of primary liver cancers recorded in cancer data banks. Globally, around 440 000 new cases of HCC occur annually, accounting for around 5.5% of all human cancer incidences [1].

Mostchemotherapeutic drugs for cancer treatment are molecules identified and isolated from plants or their effects ofseed extracts (ethanol extraction) on human tumor cells. From the dawn of ancient medicine, chemical compounds derived from plants have been used to treat human diseases. Naturalproducts have received increasing attention over the past 30 years for their potential as novel cancer preventive andtherapeutic agents[2, 3].Approximately 60% of drugs currently used for cancertreatment have been isolated from natural products [4].Interestingly, a number of non-nutrient chemicals from plants and fruits have also been reported to possess anticancer activity.Apoptosis in neoplastic cells but not in normal cells [5]. Syzygium cumini (S. cumini) (L.) fruits and seeds Skeels (BlackPlum) are edible and are reported to contain vitamin C,gallic acid, tannins, anthocyanins, including cyanidin-,petunidin, malvidin-glucoside and other components[6].*Syzygium cumini* (L.), a connatural plant medicine having multiple pharmacological actions.Acquire considerable potential value clinically. The plant has many imperative compoundswhich present the nearly all characteristics of the plant. Though many works onpharmacological activities of phytochemical constituents of *Syzygium cumini (L.)* has beencarried out, still much more is remaining to work on the development of novel drug deliverysystems of *Syzygium cumini (L.)* extract and its isolated compounds. Many active ingredients have been found in the seeds of *N. sativa*. The seeds contain both fixed and essential oils,proteins, alkaloids and Saponin.Pharmacologically important components: Thymoquinone (TQ), dithymoquinone (DTQ), thymohydroquinone (THQ), and Thymol (THY), in the oil of *N. sativa* seed by HPLC. Much of the biological activities of the seeds have been shown to be due toThymoquinone, the major component of the essential oil, which is also present in the fixed oil[7]. TQ isconsidered as potent anti-oxidant [8], anticarcinogenic and anti-mutagenic agent [9] Moreover, TQ is a relatively safe compound, particularly whengiven orally to experimental animals [10].Alpha (α)-hederin, a pentacyclic triterpene saponin isolated from the seeds of *N. sativa*, was also reported to have potent *in vivo* antitumor activity[11].

1. MATERIALS AND METHODS

1. CO_2 incubator- Sanyo, Japan. 2. Multimode micro plate reader-Biotech, USA. 3. Refrigerated centrifuge- Remi, India. 4. Cell: HepG2 cell line - NCCS Pune. 5. MTT, (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide)) from Sigma. 6. Fetal bovine serum from Genetic Biotech, India.7. Trypsin from SRL Chemicals. 8. Penicillin/Streptomycin from Sigma. 9. DMEM medium from Genetix Biotech, India.10. DMSO from SRL chemicals.

2. CELL CULTURE AND MTT ASSAY PROCEDURE:

1. The liver hepatocellular carcinoma, HepG2 cell line was purchased from NCCS Pune. The cells were grown in a DMEM medium supplemented with 10% fetal bovine serum and antibiotics as mentioned earlier. Cell proliferation (MTT) assay was performed following the method described by Carmichael *et al*., (1987) and percentage of cell viability was determined by spectrophotometric determination of accumulated formazan derivative in treated cells at 570 nm in comparison with the untreated controls.

2. For the MTT assay, the cells were grown in 25 cm ×25 cm ×25 cm tissue culture flasks containing DMEM medium as culture medium supplemented with 10% FCS, 100 U/ml penicillin, 100 µg/ml streptomycin (GIBCO) and grown at 37°C under a humidified atmosphere of 95% air and 5% CO_2. Cells were regularly passaged and maintained before including for the experiment.

3. When a cell density in a culture flask reached 70-80% confluence,

they were trypsinized and seeded in 96-well plates in the density of 5000 cells per well in 100 µL and incubated for 24 hours at CO2 incubator.

4. Next day, test item was prepared as 10 mg/ml stocks by adding directly in to phosphate buffered saline, PBS (1.5 mM KH_2PO_4, 6.5 mM Na_2HPO_4, 137 mM NaCl, 2.7 mM KCl. pH 7.4),. The working stock of 2X (2000, 600, 200, 60, 20, 6, and 2 µg/ml) concentration to the cell in 100 µL volume and the final concentration range were: 1000, 300, 100, 30, 10, 3, and 1 µg/ml. 100 µL of diluted stocks were added to the cell and the plate was further incubated for 48 hours in the CO2 incubator at 37°C.

5. MTT solution was composed of 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) at 5 mg/ml in PBS. From this solution 50 µL was pipette out into each well to achieve 1mg/mL as final concentration. The plate was further incubated for 3 hours in incubator and the medium was carefully decanted. The formazan crystals were air dried in dark place and dissolved in 100 µL DMSO and the plates were mildly mixed at room temperature and the OD was measured using Synergy HT micro plate reader at 570 nm.

6. From the optical densities the percentage growths were calculated using the following formula:

Percentage growth= 100× [(T-T0)/(C-T0)]

Where, T is optical density of test, C is the optical density of control, T0 is the optical density at time zero (at the time of compound addition will serve as blank to assess the cytotoxicity). From the percentage growths a dose response curve was generated and GI_{50} values were calculated.

3. CELL IMAGING:

After 48 hours before adding MTT solution treated cells were observed under microscope for cell morphology analysis and images of each concentration was captured and recorded.

RESULTS

4. CELL GROWTH INHIBITION PROPERTY

The test items *S. cumini* and *N.sativa* were tested against HepG2 cell line. The test items concentration ranging from 1000, 300, 100, 30, 10, 3, and 1 µg/ml in semi logarithmic range. The test compound of each concentration was performed in Triplicate and cumulative variation were maintained less than 20% between the data points.

The test compounds are not showing much cytotoxic activity in HepG2 cell lines. Both compounds are exhibiting the GI_{50} values are more than 1mg/ml. between the test compounds, the compound *S.cumini* exhibiting slight more cytotoxicity than *N. sativa* but the activity is not statistically significant. Results and raw data have been illustrated in the following table and graph.

4.1 Table: 1 Raw Data Absorbance values at 570nm against HepG2 and percentage growth inhibition

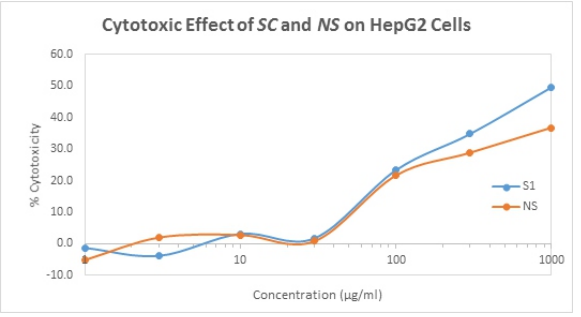
Reading at 570 nm	Sample concentration (µg/ml)	*S.cumini*			*N.sativa*		
	1000	1.574	1.684	1.617	1.793	1.894	1.845
	300	1.899	1.808	1.928	2.021	2.023	1.896
	100	2.099	2.098	2.028	2.121	2.123	2.066
	30	2.488	2.387	2.459	2.55	2.306	2.526
	10	2.435	2.306	2.521	2.55	2.406	2.326
	3	2.699	2.404	2.518	2.312	2.562	2.449
	1	2.408	2.532	2.552	2.562	2.485	2.643
	Untreated Control	2.593	2.412	2.396	2.513	2.396	2.539

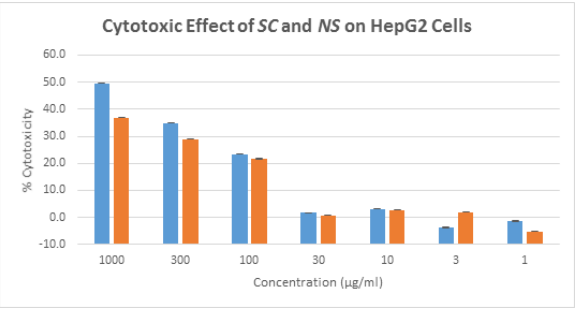
T0 : 0.763	1000	0.811	0.921	0.854	1.03	1.131	1.082
At the time of	300	1.136	1.045	1.165	1.258	1.26	1.133
compound addition	100	1.336	1.335	1.265	1.358	1.36	1.303
	30	1.725	1.624	1.696	1.787	1.543	1.763
	10	1.672	1.543	1.758	1.787	1.643	1.563
	3	1.936	1.641	1.755	1.549	1.799	1.686
	1	1.645	1.769	1.789	1.799	1.722	1.88
	Untreated Control	1.83	1.649	1.633	1.75	1.633	1.776

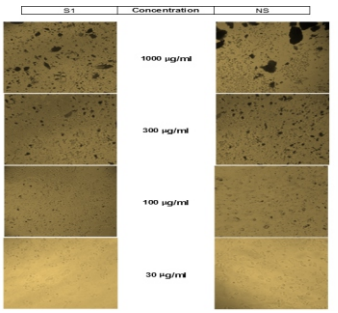
% Cell Viability	1000	47.4	53.8	49.9	60.2	66.1	63.2
	300	66.4	61.0	68.1	73.5	73.6	66.2
	100	78.0	78.0	73.9	79.3	79.4	76.1
	30	100.8	94.9	99.1	104.4	90.1	103.0
	10	97.7	90.1	102.7	104.4	96.0	91.3
	3	113.1	95.9	102.5	90.5	105.1	98.5
	1	96.1	103.3	104.5	105.1	100.6	109.8
	Untreated Control	106.9	96.3	95.4	102.2	95.4	103.7

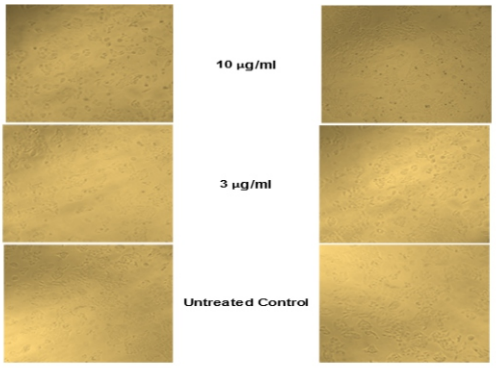
% Cell Death	1000	52.6	46.2	50.1	39.8	33.9	36.8
	300	33.6	39.0	31.9	26.5	26.4	33.8
	100	22.0	22.0	26.1	20.7	20.6	23.9
	30	-0.8	5.1	0.9	-4.4	9.9	-3.0
	10	2.3	9.9	-2.7	-4.4	4.0	8.7
	3	-13.1	4.1	-2.5	9.5	-5.1	1.5
	1	3.9	-3.3	-4.5	-5.1	-0.6	-9.8
	Untreated Control	-6.9	3.7	4.6	-2.2	4.6	-3.7

Average of % Cell Death	Concentration (ug/ml)	SC	NS
	1000	49.6	36.9
	300	34.8	28.9
	100	23.4	21.7
	30	1.8	0.8
	10	3.2	2.8
	3	-3.8	2.0
	1	-1.3	-5.2
	Untreated Control	0.5	-0.5









S1 (S.cumini-SC), NS (N.sativa-NS)

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