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Cytotoxic Activity of Ayurvedic Polyherbal Product Muneks On Selected Cell Lines

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Abstract

In-vitro cytotoxicity of Muneks tablet was tested by MTT for A549 (Human Lung Carcinoma), HeLa (Human Cervix Adenocarcinoma), SKOV3 (Human ovarian cancer), CaCo2 (Human Colon carcinoma), MDA-MB 231 (Human breast cancer), JURKAT 3 (Human leukemia), HL-60 (Human leukemia) and LN Cap (Human prostate Adenocarcinoma) cell lines. Muneks tablet was taken at concentrations ranging from 1000 µg/ml to 7.8µg/ml to determine the percentage growth inhibition on the cell lines A549, HeLa, SKOV3, CaCo2, MDA MB 231, JURKAT, HL-60 and LN Cap. The Muneks exhibited a CTC50 value of 117.71±2.37, 202.39±4.25, 94.28±2.06, 155.86±1.03, 167.47±4.48, 277.03±3.15, 312.81±4.78 and 231.51±3.61 respectively.

Keywords
Muneks, cytotoxicity, MTT, anticancer.

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1. INTRODUCTION:

Cancer is one of the most dreaded diseases of the 20th century and spreading further with continuance and increasing incidence in 21st century[1]. It is considered as an adversary of modernization and advanced pattern of socio-cultural life dominated by Western medicine. Recent studies have shown that conventional chemotherapy has several limitations due to its adverse effects at physical, mental, emotional, social and economic levels. Multidisciplinary scientific investigations are making best efforts to combat this disease, but the sure-shot, perfect cure is yet to be brought into world medicine[2]. Even in the case of herbal products there are negligible scientifically validated anti-cancer preparations. Standard quality assured method to produce an effective and safe product against cancer is lacking. Muneks is a polyherbo-mineral fortified tablet. It is prepared based on the description given in the ancient texts of Ayurveda, but with unique methodology to get best possible pharmacological effect. Here an attempt is made to validate the anticancer effect of Muneks tablet by evaluating its in vitro cytotoxicity activity on selected cell lines.

1.1. OBJECTIVE:

The purpose of this Study is to evaluate the Muneks for its cytotoxicity against selected cell lines.

2. MATERIALS AND METHODS:

2.1. Material: Muneks tablet is a polyherbomineral Ayurvedic product designed and developed by Muniyal Ayurveda Research Centre Manipal as per the GMP guidelines following strict quality control protocol[3]. Ingredients of the product and quality test report are as shown in Table1 and Table 2 respectively.

Table1. Composition of Muneks Tablet, each 500 mg tablet is containing:

No	Sanskrit Name	Part used	Latin/English name	Quantity
1	Punarnava	Dried root	*Boerhavia diffusa*	32 mg
2	Vasa	Dried root	*Adhatoda vasica*	36 mg
3	Sadapushpa	Dried leaves	*Vinca rosea*	24 mg
4	Kanchanara	Dried stem bark	*Bauhinia variegata*	28 mg
5	Guggulu	Purified Oleo gum resin	*Commiphora mukul*	32 mg
6	Nimba	Dried stem bark	*Azadirachta indica*	08 mg
7	Ativisha	Dried root	*Aconitum heterophyllum*	08 mg
8	Ishwari	Dried root	*Aristolochia indica*	24 mg
9	Madhusnuhi	Dried root	*Smilax china*	08 mg
10	Bhrngaraja	Dried whole plant	*Eclipta alba*	24 mg
11	Guduchi Satva	Starch extract of stem	*Tinospora cordifolia*	12 mg
12	Ashvagandha	Dried root	*Withania somnifera*	12 mg
13	Bala	Dried root	*Sida cordifolia*	12 mg
14	Hareetakee	dry fruits	*Terminalia chebula*	04 mg
15	Vibhitaki	dried fruits	*Terminalia bellerica*	04 mg
16	Amalaki	dried fruits	*Emblica officinalis*	16 mg
17	Shilajatu	Fossil resin	*Asphaltum punjabicanum*	12 mg
18	Guduchi	Dried stem	*Tinospora cordifolia*	08 mg
19	Pippali	Dried fruit	*Piper longum*	04 mg
20	Maricha	Dried fruit	*Piper nigrum*	04 mg
21	Shunthi	Dried rhizome	*Zingiber officinalis*	04 mg
22	Shigru	Dried stem bark	*Moringa oleifera*	24 mg
23	Tulasi	Dried leaves	*Ocimum sanctum*	16 mg
24	Haridra	Dried rhizome	*Curcuma longa*	24 mg
25	Vanga Bhasma	Incinerated tin	Stanni oxidum	06 mg
26	Yashada Bhasma	Incinerated zinc	Zinci oxidum	06 mg
27	Swarna Makshika bhasma	Incinerated copper pyrite	*Oxidum copper pyrite*	12 mg
28	Abhraka Bhasma	Incinerated mica	*Mica oxidum*	12 mg
29	Loha bhasma	Mineral	Incinerated Iron(ferric oxide)	12 mg
30	Pravala bhasma	Mineral	Coral calx(calcium carbonate)	12 mg
31	Excipient	Gum	*Gum acacia*	40 mg

Table 2 Physicochemical analysis of test drug

Parameters	**Sample value**
Loss on drying	2.44 %
Tablet disintegration test	3-5 minutes
Tablet Hardness test	4.0 kg/cm
Uniformity of weight	500 ± 2.5 mg
pH value	6.1
Total ash content	18%
Acid insoluble ash value	1.2 %
Water soluble ash value	16.5 %
Alcohol extractive value	6.4 %
Water soluble extractive value	4.4 %
Chloroform soluble extractive value	9.36%

2.2. Method:

2.21. Outline of the method[4]

The *in vitro* cytotoxicity was performed for Muneks Tablets on A549 (Human Lung Carcinoma), HeLa (Human Cervix Adenocarcinoma) SKOV3 (Human ovarian cancer), CaCo2 (Human Colon carcinoma), MDA MB 231 (Human breast cancer), JURKAT

(Human leukemia), HL-60(Human leukemia), and LN Cap (Human prostate Adenocarcinoma) Cell lines to find toxic concentration of the Muneks Tablet by MTT assay.

2.22. Preparation of test solution

For cytotoxicity studies, 10mg of the test substance was separately dissolved and volume was made up with MEM / DMEM-HG / RPMI supplemented with 2 % inactivated FBS to obtain a stock solution of 1 mg/ml concentration and sterilized by 0.22µ syringe filtration. Serial two fold dilutions were prepared from this for carrying out cytotoxic studies.

2.23. Cell line and culture medium

A549 (Human Lung Carcinoma), HeLa (Human Cervix Adenocarcinoma), SKOV3 (Human ovarian cancer), $CaCO_2$ (Human Colon carcinoma) MDA MB 231 (Human breast cancer) JURKAT (Human leukemia), HL-60 (Human leukemia), and LN Cap (Human prostate Adenocarcinoma) Cell lines were procured from National Centre for Cell Sciences (NCCS), Pune, India. Stock cells were cultured in their respective media viz., MEM/DMEM-HG/RPMI supplemented with 10% inactivated Fetal Bovine Serum (FBS), penicillin (100 IU/ml), streptomycin (100 g/ml) and amphotericin B (5 g/ml) in a humidified atmosphere of 5% CO2 at 37C until confluent. The cells were dissociated with TPVG solution (0.2% trypsin, 0.02% EDTA, 0.05% glucose in PBS). The stock cultures were grown in 25 cm^2 culture flasks and all experiments were carried out in 96 well microtitre plates (Tarsons India Pvt. Ltd., Kolkata, India).

2.24. Cytotoxicity studies

In all the cell lines, the monolayer cell culture was trypsinized and the cell count was adjusted to 100,000 cells/ml using respective media viz., MEM/DMEM-HG/RPMI containing 10% FBS. To each well of the 96 well microtitre plate, 0.1 ml of the diluted cell suspension was added. After 24 h, when a partial monolayer was formed, the supernatant was flicked off, monolayer washed once with medium and 100 l of different test concentrations of Muneks were added on to the partial monolayer in microtitre plates. The plates were then incubated at 37°C for 72 h in 5% CO_2 atmosphere, and microscopic examination was carried out and observations were noted at every 24 h interval.

2.25. MTT assay

After 72 h incubation, the drug solutions in the wells were discarded and 50µl of MTT in PBS was added to each well. The plates were gently shaken and incubated for 3 h at 37°C in 5% CO2 atmosphere. The supernatant was removed and 100 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 540 nm. The percentage growth inhibition was calculated using the standard formula and concentration of Muneks needed to inhibit cell growth by 50% (CTC50) values was generated from the dose-response curves for each cell line.

3. RESULTS AND DISCUSSION:

The Muneks Tablet was tested for *in vitro* cytotoxicity studies against A549 (Human Lung Carcinoma), HeLa (Human Cervix Adenocarcinoma), SKOV3 (Human ovarian cancer), CaCo2 (Human Colon carcinoma), MDA MB 231 (Human breast cancer), JURKAT (Human leukemia), HL-60(Human leukemia), and LN Cap (Human prostate Adenocarcinoma) cells by MTT assay exposing the cells to different concentrations of test substance. The Muneks was taken at concentrations ranging from 1000 µg/ml to 7.8 µg/ml to determine the percentage growth inhibition on the cell lines A549, HeLa, SKOV3, CaCo2, MDA- MB 231, JURKAT, HL-60, and LN Cap. The test substance Muneks Tablet exhibited a CTC50 value of 117.71±2.37, 202.39±4.25, 94.28±2.06, 155.86±4.03, 167.47±4.48, 277.03±3.15, 312.81±4.78, and 231.51±3.61 on A549, HeLa SKOV3, CaCo2, MDA-MB 231, JURKAT, HL-60, and LN Cap cell lines respectively. (Tables 3 to 10 and Figures 1 to 8).

Some ingredients of the test drug Muneks have proven anticancer activity where as some other ingredients showed antioxidant effects where they acted as potent inhibitors of lipid peroxide formation and scavenger of hydroxyl and super oxide radicals in vitro. In a study carried out by Meghna T. Adhvaryu et.al, author evaluated anti-tumor activity and chemo-preventive potential of Ayurvedic herbs viz. Curcuma longa L., Ocimum sanctum L., Tinospora cordifolia (Wild) [Miers ex Hook F. & Thomas] using Dalton Lymphoma ascites (DLA) tumor model in Swiss Albino mice. All these herbs were found to be effective[5]. In a study carried out by S.N. Gaidhani et.al. *Picrorhiza kurroa* and *Piper longum* showed promising anti-cancer potential against colon cancer cell lines whereas studies have indicated that

Withaferin A present in Ashvagandha possesses potent anti-cancer activity[6]. Studies carried out on extracts of *Withania somnifera* and *Tinospora cordifolia* against cell lines of breast cancer and cervical cancer have reported their potential cytotoxic and apoptosis activities against these cancers[7]. In another study Withania somnifera was found to be effective against lungs cancer (Hop62) and Leukemia (K562) cell lines[8]. In a study it has been noted that *Zingiber officinale* supplementation suppressed liver carcinogenesis due to free radical scavenging activity[9]. Polyphenolic compounds present in *Emblica officinalis* juice showed strong inhibition against B16F10 cancer cell growth[10]. *Bharali and colleagues* have shown that *Moringa oleifera* has activity against chemical carcinogens via hepatic pathway[11]. Isolated compounds from Neem have shown impressive efficacy against a wide variety of human cancer cell lines, and animal models for human cancers that include colon, stomach, Ehrlich's carcinoma, lung, liver, skin, oral, breast and prostate cancers[12]. In total it can be said that Muneks containing all these ingredients exhibits a promising anticancer efficacy.

Table 3: Cytotoxic properties of Muneks against A549 cell line

Sl. No	Test Conc. (µg/ml)	% Cytotoxicity	CTC50 (µg/ml)
1.	1000	84.02±0.30	117.71±2.37
2.	500	70.08±1.81	
3.	250	63.84±0.54	
4.	125	52.02±0.78	
5.	62.5	34.90±0.72	
6.	31.25	30.90±0.58	
7.	15.6	27.52±0.48	
8.	7.8	21.36±0.77	

Table 4: Cytotoxic properties of Muneks against HeLa cell line

Sl. No	Test Conc. (µg/ml)	% Cytotoxicity	CTC50 (µg/ml)
1.	1000	77.33±2.33	202.395±4.24
2.	500	69.29±4.40	
3.	250	55.12±0.65	
4.	125	41.04±0.39	
5.	62.5	34.00±1.29	
6.	31.25	18.37±0.65	
7.	15.6	12.52±0.65	
8.	7.8	4.39±0.52	

Table 5: Cytotoxic properties of Muneks against SKOV3 cell line

Sl. No	Test Conc. (µg/ml)	% Cytotoxicity	CTC50 (µg/ml)
1.	1000	70.15±0.10	94.284±2.06
2.	500	66.67±0.39	
3.	250	60.79±0.19	
4.	125	55.60±0.77	
5.	62.5	44.47±0.10	
6.	31.25	42.62±0.39	
7.	15.6	41.12±0.39	
8.	7.8	39.07±0.19	

Table 6: Cytotoxic properties of Muneks against CaCO2 cell line

Sl. No	Test Conc. (µg/ml)	% Cytotoxicity	CTC50 (µg/ml)
1.	1000	69.92±0.70	155.86±4.03
2.	500	66.08±0.41	
3.	250	54.69±0.90	
4.	125	48.44±0.52	

5.	62.5	38.22±1.49	
6.	31.25	25.00±2.40	
7.	15.6	20.51±0.90	
8.	7.8	15.23±1.19	

Table 7: Cytotoxic properties of Muneks against MDA MB 231 cell line

Sl. No	Test Conc. (µg/ml)	% Cytotoxicity	CTC50 (µg/ml)
1.	1000	68.73±1.64	167.47±4.48
2.	500	62.26±0.71	
3.	250	56.02±0.34	
4.	125	46.90±0.36	
5.	62.5	42.63±0.34	
6.	31.25	38.05±0.56	
7.	15.6	33.92±0.54	
8.	7.8	29.83±2.29	

Table 8: Cytotoxic properties of Muneks against JUKART 3 cell line

Sl. No	Test Conc. (µg/ml)	% Cytotoxicity	CTC50 (µg/ml)
1.	1000	75.37±0.40	**277.031±3.15**
2.	500	70.16±0.80	
3.	250	47.55±0.33	
4.	125	40.73±1.06	
5.	62.5	38.72±1.96	
6.	31.25	35.05±0.73	
7.	15.6	30.74±0.97	
8.	7.8	17.71±0.60	

Table 9: Cytotoxic properties of Muneks against HL-60 cell line

Sl. No	Test Conc. (µg/ml)	% Cytotoxicity	CTC50 (µg/ml)
1.	1000	87.04±0.58	**312.81±4.78**
2.	500	59.37±0.33	
3.	250	46.16±0.33	
4.	125	38.53±0.44	
5.	62.5	34.76±0.77	
6.	31.25	30.61±1.17	
7.	15.6	16.65±1.52	
8.	7.8	9.22±0.26	

Table 10: Cytotoxic properties of Muneks against LN-CAP cell line

Sl. No	Test Conc. (µg/ml)	% Cytotoxicity	CTC50 (µg/ml)
1.	1000	84.01±0.30	231.512±3.61
2.	500	70.06±1.81	
3.	250	51.00±0.11	
4.	125	44.12±0.81	
5.	62.5	34.29±0.73	
6.	31.25	30.29±0.59	
7.	15.6	26.84±0.48	
8.	7.8	20.61±0.77	

Figure 1. Anticancer effect of Muneks tablets (MTT assay) on A549 cell line

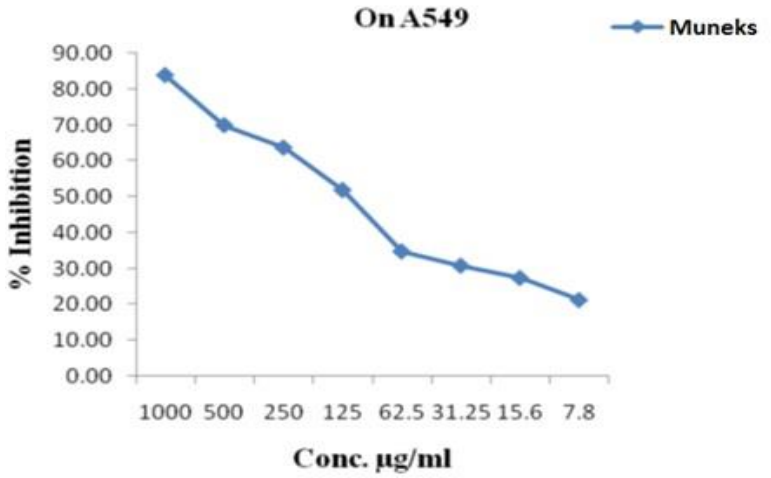


Figure 2. Anticancer effect of Muneks tablets (MTT assay) on HeLa cell line

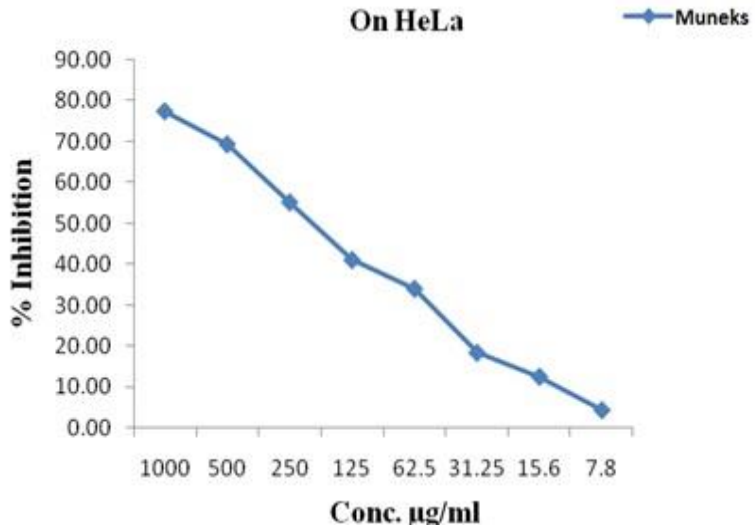


Figure 3. Anticancer effect of Muneks tablets (MTT assay) on SKOV3 cell line

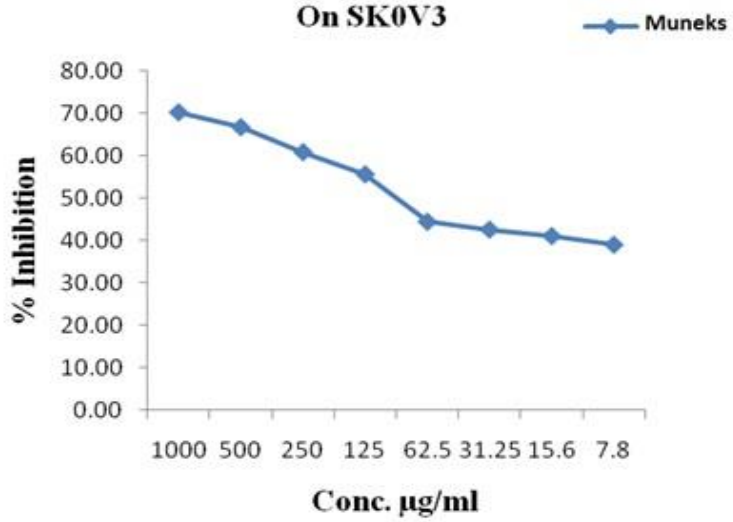


Figure 4. Anticancer effect of Muneks tablets (MTT assay) on CaCO2 cell line

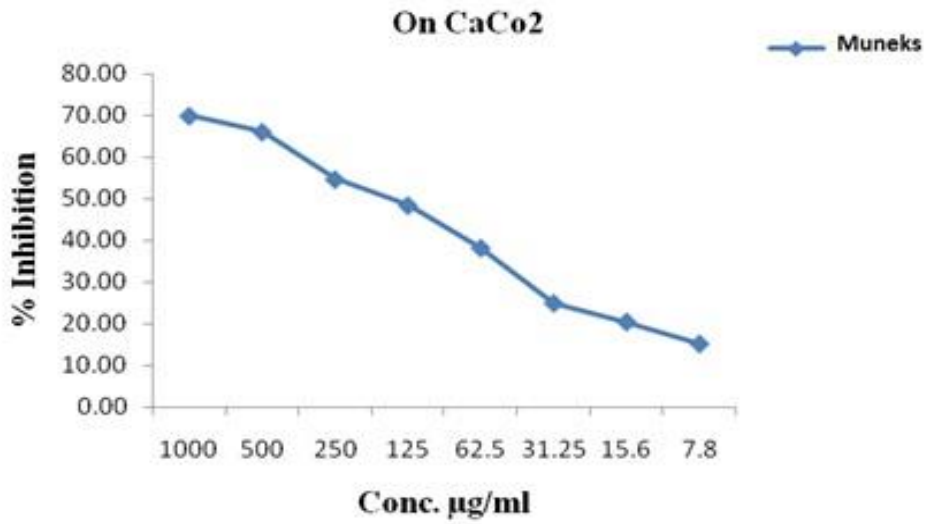


Figure 5. Anticancer effect of Muneks tablets (MTT assay) on MDA MB 231 cell line

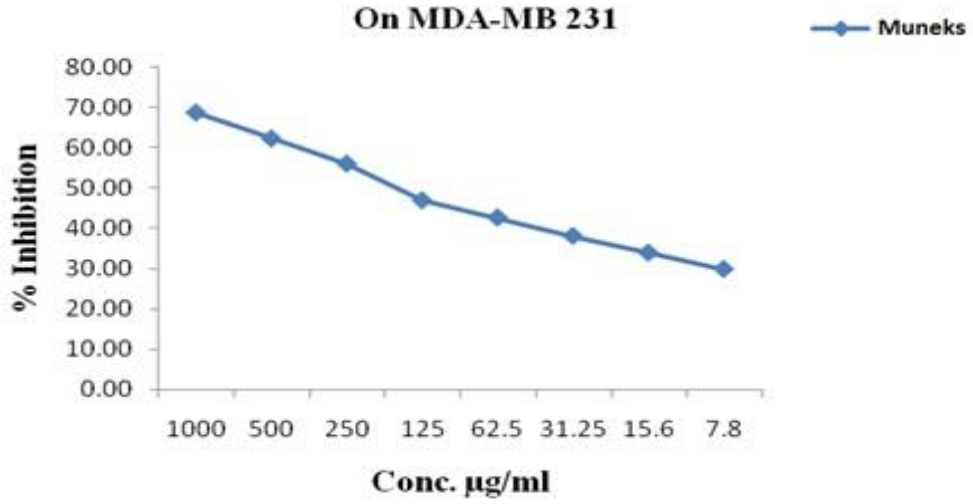


Figure 6. Anticancer effect of Muneks tablets (MTT assay) on JUKART cell line

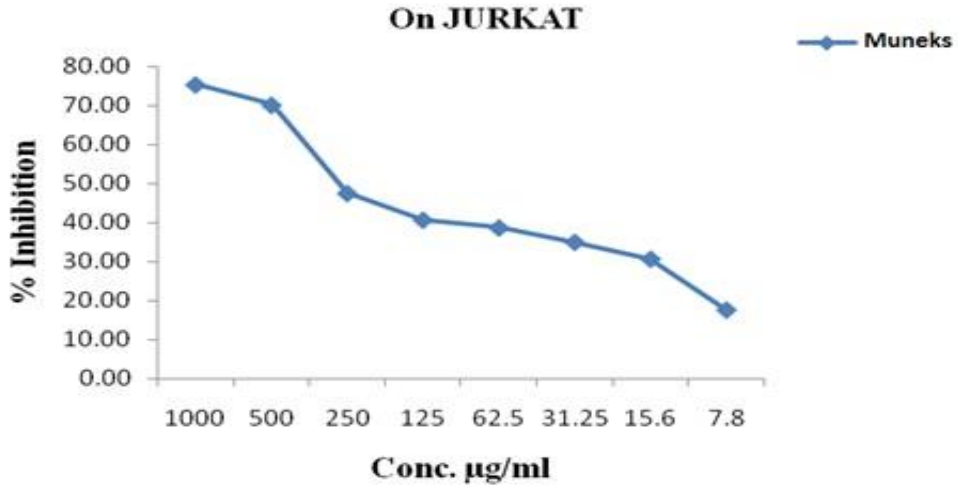


Figure 7. Anticancer effect of Muneks tablets (MTT assay) on HL-60 cell line

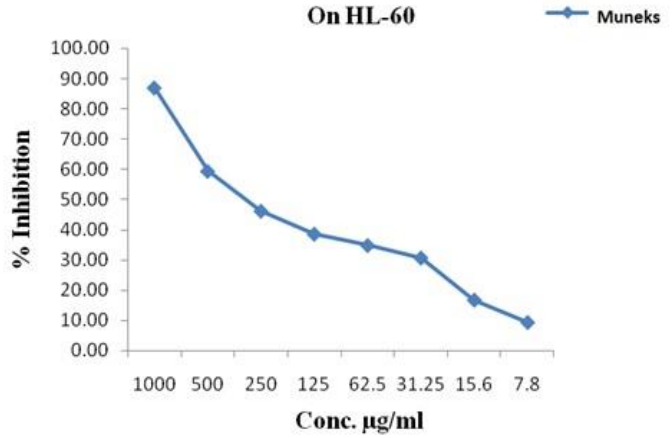
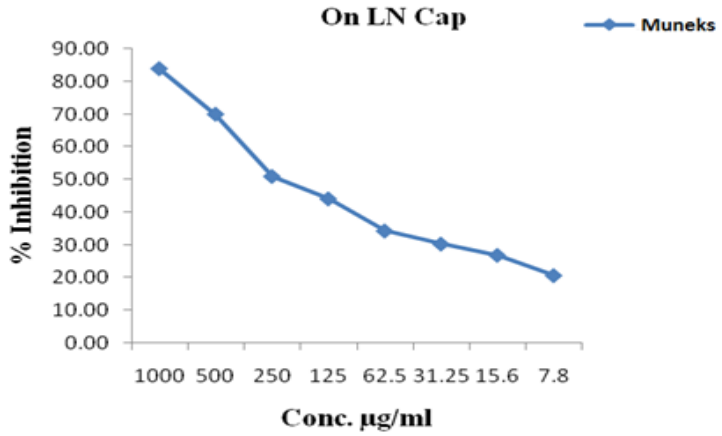


Figure 8. Anticancer effect of Muneks tablets (MTT assay) on LN Cap cell line



4. CONCLUSION:

Test drug Muneks was found to possess considerable cytotoxic effect against the selected A549 (Human Lung Carcinoma), HeLa (Human Cervix Adenocarcinoma), SKOV3 (Human ovarian cancer), CaCo2 (Human Colon carcinoma) MDA MB 231 (Human breast cancer) JURKAT (Human leukemia), HL-60 (Human leukemia), and LN Cap (Human prostate Adenocarcinoma) cell lines suggesting it as a promising anticancer drug.

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CONFLICT OF INTEREST STATEMENT:

Author declares no conflict of interest.

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