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Original Article

Mebendazole: Easy reachable and ubiquitous target for breast cancer cell lines

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ABSTRACT

Breast cancer is the most commonly diagnosed malignancy worldwide and lamentably there was no perfect chemotherapy option that can diminish the mortality rate because of breast cancer complexities. Along these lines, analyst ought to more concentration looking for a perfect chemotherapeutic drug that is effective and give secure treatment against tumor. At present, several novel therapeutic targets such as Mebendazole, Metformin and COX-2 inhibitors attracted significance because of same reason. The primary aim of this research project was to evaluate the cytotoxicity activity of Mebendazole against breast cancer cell lines in –vitro. For this purpose, we used four cancerous cell lines including MCF-7, MDA-MB-231, HT-29 and Hela cell lines and six different dilutions of Mebendazole ranges between2-110. As a single operator, impacts of Mebendazole on MTT test (cytotoxicity assay) delighted that Mebendazole can adequately diminished the % viability of cancerous cell lines with mean IC50 were 7.449 ± 0.535, 7.68± 0.442, 22.36 ± 4.315 and 849.12 ± 23.96 in MCF-7, MDA-MB-231, Hela and MCF-10 cell lines respectively. This demonstrates Mebendazole can adequately represses their progress in-vitro as alone therapy.

Key words: MTT assay, MCF-7, MCF-10, MDA-MB-231, Hela, HT-29 cell lines, Trypan blue dye exclusion assay.

INTRODUCTION

Breast cancer is the second most commonly diagnosed cancer among women and in the USA nearly 182,000 women spotted with breast cancer annually which is an accounting for approximately 26% of all cancers among women. Each year, 40,000 women die due to breast cancer related complications, making it the second-leading reason of cancer deaths among American females just next to lung cancer[i]. As per available statistics, about 1.38 million (23% per year) women annually suffer from cancer diseases around the world, out of which about 458,000 women die annually only due to breast cancer which is about 14% of cancer related deaths. However, it is interesting to know that breast cancer incidences are more common in developed countries but death rates are higher in developing countries[ii]. Most of the cancer patients die due to metastatic problems and unfortunately at that stage even chemotherapy couldn't be helpful to decreases the death incidents. That is why, nowadays researchers should more focus on novel options of drugs that can itself have cytotoxicity and can effectively synergize the effects of effective chemotherapy agents[iii].

Microtublues play diversity of function in human cells as maintaining the cellular morphology, cellular migration, proper chromosomal segregation and mitosis. These diversities make Microtubules as attractive target for anti-cancerous drugs[iv]. Mebendazole which most commonly prescribed economical anthelmintic agent, this agent can exert their effects by binding with beta subunit of tubulin and thus prevent the polymerization of tubulin into microtubules and can also interfere with glucose transport of helminths[v].

Recently it was found that Mebendazole in addition to their primary effects of anti-parasitic can effectively targeted the tumor prone cells and can effectively decreases the mitosis by disrupting the mitotic spindle and cellular arrest at G2/M phase because of inhibition of mitosis and eventually apoptosis ensues. Furthermore Mebendazole can directly **induce** the apoptosis of tumor cells by

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inhibiting the expression of antiapoptotic protein, Bcl-2, thus this unable to interacts with proapoptotic protein such as BAX, which ultimately leading to activation of caspase pathway and ensues cellular apoptosis[vi].

Apart from their prime actions on mitotic spindle Mebendazole can declines the angiogenesis by diminutions of expression of vascular endothelial derived growth factor (VEDGF), Epidermal derived growth factor (EDGF). Thus by inhibiting the neovascularization Mebendazole can famish the tumor cells and ultimately cellular death occurs due to cellular starvation[vii].

Correspondingly, Mebendazole can squander the cellular energy reserve by uncoupling of oxidative phosphorylation at mitochondria. Additionally, as an add-on therapy, Mebendazole can effectively synergize the effects of standard chemotherapeutic agents by reducing their resistance by decreasing the impact of efflux pump (P-glycoprotein)[viii].

The above proposed anticancerous mechanisms of Mebendazole fundamental objective of this trial was to determine and to analyze the in-vitro antitumor action of Mebendazole on various cancerous cells essentially on breast cancer cells as there was insufficient supporting trial for antitumor movement on breast cancer cell lines.

MATERIAL AND METHODS

For assessing the in-vitro cytotoxicity of Mebendazole against cancerous cells we were using five cell lines including MCF-7, MDA-MB-231, HT-29, Hela and MCF-10, out of which first four were cancerous and last one was normal epithelial cell breast (to assessed the selectivity index of Mebendazole against breast cancer).

Cells cultured (both cancer cells and of nonmalignant cells) were treated with different dose ranges of the Mebendazole solutions starting from the lowest dose and incubated for 48-72 hours. Then cell growth inhibition were assessed by 3- (4, 5-dimethyl thiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) assay which was cytotoxicity assay. Additionally for MCF-7 cell line cytotoxicity of Mebendazole can be assessed by trypan blue dye exclusion assay. For these assays readings with each dose were repeated four times in four separate days as described by Florento et al., (2012)[ix].

MTT assay was colorimetric assay which usually measures the proliferation rate because whenever due to events like necrosis or apoptosis resulting in reduction of cellular viability. The foremost of this test is to evaluate the limit of Mitochondrial chemical succinate dehydrogenase in living cells to diminish the yellow water dissolvable substrate 3-(4, 5-dimethyl thiazol-2-yl)- 2, 5-diphenyl tetrazolium bromide (MTT) into an insoluble, shaded formazan item which is measured spectrophotometrically. Meanwhile reduction of MTT can only occur in metabolically active cells, the level of activity is a measure of the viability of the cells[x].

The percentage of viability evaluated by comparing the absorbed values of samples at particular wavelength (570nm) that have cell line along with test drug and reagent and samples that have merely cell line with reagent without any drug solutions. Absorbed values were progressively decreasing with the increase in cellular inhibition[xi].

Trypan blue dye exclusion assay was another widely used viability assay that usually used for evaluating the amount of viable cells within the cellular suspension. It is based on principle that viable cells that restrain the entrance of certain dye such as trypan blue and propidium inside the cells due of their intact cell membrane conversely dead cells do not as the integrity of cellular membrane is no longer maintain in death cells. Accordingly in this assay, cell suspension was chiefly mixed with dye and then visually examined to conclude whether cells take up or reject entry of dye within the cell. Thus a viable cell will have a clear cytoplasm because of their intact membrane exclude dye whereas a nonviable cell will have a blue cytoplasm[xii].

The IC50 (Cell inhibition 50%) value designates the drug concentration obligatory to diminish the number or fraction of cells to 50% as compared with the controls. IC50 esteem is the focal execute for assessing and likening the after effects of novel anti-cancerous drugs with standard medication[xiii].Selectivity index of drug calculated by dividing the IC50 value in the nonmalignant cells by that in the cancerous cells[xiv].

Statistical Analysis: The data was analyzed on IBM SPSS version 24.0 and the results were presented as mean, standard deviation, minimum and maximum was reported for every response variable like Ab, At, Ac, %, and Fa for all the doses among cell types. Percentage decrease of At and percentage viability were calculated by amount of decrease divided by initial value and then multiply by 100 (amount of decrease was calculated by initial value of dose 0 minus value that have to compare with initial value from dose 1 to dose 6) . Statistical test, Kruskul-Wallis was performed to estimate the mean difference of same response

variables among doses of Mebendazole. A p-value of 0.05 or less was considered as statistically significant and highly significant at 0.01 or less.

RESULTS

Comparison of effects of different doses of Mebendazole on MCF-7 cell line as assessed by effects on MTT variables shows that there were no significant differences on Ab value among the different doses of Mebendazole, $\chi2(2)$= 06.074, p=0.415 as well as for Ac from dose 0 to sixth dose of Mebendazole ,$\chi2(2)$= 2.879, p=0.824. However there was statistically highly significant difference found for At value between the different doses of Mebendazole , $\chi2(2)$= 25.660, p=<0.001 with mean At value 0.26 ± 0.01for dose 0 which decreases to 0.083 ± 0.02 for dose 6 with average percentage decrease was about -68.97 from no dose to 6th dose. Similarly for % viability there were highly significant differences were noted among the different doses of combination therapy, $\chi2(2)$= 26.276, p=<0.001 with mean viability % was 99.7 ± 0.2 for dose 0 which decreases up to 30.67 ± 5.8 with dose 6 and average percentage decrease was about -69.32 as depicted in table 1.

Correspondingly for MDA-MB-231 cell line comparison of effects of different doses of Mebendazole on MTT variables showed that there were no significant differences on Ab value among the different doses of Mebendazole, $\chi2(2)$= 02.347, p=0.885 similarly for Ac from dose 0 to dose 6 of Mebendazole, $\chi2(2)$= 1.318, p=0.971 . Although there was a statistically significant change was observed for At value between the different doses of Mebendaozle , $\chi2(2)$= 25.534, p=0.001 with mean At value 0.34 ±0.023for dose 0 which decreases to 0.17 ± 0.024for dose 6 with average percentage decrease was about -50.33 from dose 0 to 6th dose. Similarly for % viability there was highly significant differences were noted among the different doses of combination therapy, $\chi2(2)$= 26.254, p=<0.001 with mean viability % was 99.83 ± 0.21for dose 0 which decreases upto 49.07 ± 4.38 with dose 6 and average percentage decrease was about -50.84. Correspondingly, for fa there were highly significant differences noted among the different doses of Mebendazole, $\chi2(2)$=26.254, p=<0.001 with mean 0.002 ± 0.0007 value for dose 0 which increases up to 0.51 ± 0.04 for dose 6, as illustrated in table 2.

However on HT-29 human colorectal adenocarcinoma cell line there were no significant differences on Ab value among the different doses of Mebendazole, $\chi2(2)$= 01.068, p=0.983 equally for Ac from dose 0 to sixth dose of Mebendazole, $\chi2(2)$= 1.680, p=0.947. **But statistically** there was a significant change was observed for At value between the different doses of **Mebendazole**, $\chi2(2)$= 18.680, p=0.005 with mean At value 0.33 ±0.036 for dose 0 which decreases to 0.183 ±0.046for dose 6 with average percentage decrease was about -45.018 from no dose to 6th dose. Equally for % viability there was highly significant differences were noted among the different doses of Mebendazole, $\chi2(2)$= 25.618, p=<0.001 with mean viability % was 99.76 ± 0.20 for dose 0 which decreases upto 55.11 ± 7.87 with dose 6 and average percentage decrease was about -44.754. Correspondingly for fa there were highly significant differences noted among the different doses of Mebendazole, $\chi2(2)$=25.618, p=<0.001 with mean 0.002 ± 0.002 value for dose 0 which increases up to 0.45 ± 0.079for dose 6 as depicted in table 03.

Similarly for Hela cell line there were statistically non-significant differences on Ab value among the different doses of Mebendazole, $\chi2(2)$= 07.176, p=0.305in the same way for Ac from dose 0 to sixth dose of Mebendazole, $\chi2(2)$= 1.299, p=0.972. Statistically, there was a significant change perceived for At value among the different doses of Mebendazole , $\chi2(2)$= 24.775, p=<0.001 with mean At value 0.393 ± 0.014for dose 0 which decreases to 0.25 ± 0.012for dose 6 with average percentage decrease was about -36.356 from no dose to 6th dose. Likewise for % viability there was highly significant differences noted among the different doses of Mebendazole, $\chi2(2)$= 26.497, p=<0.001 with mean viability % was 99.79 ± 0.103 for dose 0 which decreases upto63.144 ± 0.90with dose 6 and average percentage decrease was about -36.722. Correspondingly, for fa there were highly significant differences noted among the different doses of Mebendazole, $\chi2(2)$=26.49, p=<0.001 with mean 0.021 ± 0.001 value for dose 0 which increases up to 0.37 ± 0.09for dose 6 as depicted in table 4.

For MCF-10 cell line as there were no significant differences on Ab value among the different doses of Mebendazole, $\chi2(2)$= 10.471, p=0.106 in the same way for Ac from dose 0 to sixth dose of Mebendazole, $\chi2(2)$= 5.735, p=0.453. Correspondingly, no significant change was observed for At value among the different doses of Mebendazole , $\chi2(2)$= 9.001, p=0.174 with mean At value 0.481 ± 0.008for dose 0 which decreases to 0.47 ± 0.009for dose 6 with average percentage decrease was only-2.71 from dose 0 to 6th dose. Likewise for % viability there was no statistically significant differences were noted among the different doses of Mebendazole, $\chi2(2)$= 10.382, p=0.109 with mean viability % was 99.77 ± 0.086for dose 0 which decreases upto98.72 ±

0.973with dose 6 and average percentage decrease was about -1.052. Harmoniously, for fa there were no significant differences noted among the different doses of Mebendazole, $\chi2(2)=10.382$, p=0.109 with mean 0.002 ± 0.0008 value for dose 0 which increases up to 0.012 ± 0.009for dose 6 as depicted in table 05.

There were significant difference noted among the IC50 values of Mebendazole among all study cell lines with $\chi2(2)= 17.429$, p=0.002. The mean IC50 value of Mebendazole in MCF-7 cancer cell line was 7.449 ± 0.535, whereas in MDA-MB-231 cell line was 7.68± 0.442, HT-29 mean IC50 value was 2.59 ± 0.218, however in hela cell mean IC50 value was 22.36 ± 4.315and finally in MCF-10 cell line mean IC50 was 849.12 ± 23.96. This showed that the lowest IC50 value of Mebendazole was observed in HT-29 human colorectal adenocarcinoma cell line. As illustrated in table 6 and figure 1.

There was a statistically non-significant difference in selectivity indices between Mebendazole treated MCF-7 and MDA-MB-231, $\chi2(2) = 1.114$, p = 0.291,with a mean selectivity index 114.865 ± 11.20for MCF-7 and 111.245 ± 8.99for MDa-MB-231. This showed that selectivity index was lower in MDA-MB -231 Metformin treated cell line. As shown in table 07 and figure 2.

Table 8 illustrated the effects of different doses of Mebendazole on different variables of Trypan blue dye exclusion assay which includes viable cell counts, total cells and % viability. There was a statistically significant difference in viable cell count between the different doses of Mebendazole, $\chi2(2) = 19.221$, p = 0.004, with mean viable cells count was 261.92 ± 1.182for dose 0 that decreases up to 160.33 ± 1.444 with sixth dose with an average percentage decrease was -38.792 from dose 0 to dose 6. Consequently, there was statistically highly significant difference was noted in death cell counts among the different doses of Mebendazole,$\chi2(2) = 19.082$, p = 0.004 with mean value of 4.683±0.913 for dose 0 that increases upto 150.90±5.23 for dose 6. Similarly there was statistically significant difference in total cell count between different doses of Mebendazole, $\chi2(2) = 12.641$, p = 0.049,mean total cells were 267.7 ± 1.226with dose 0 and 263.24± 1.029 with dose 6. Equally highly significant differences were noted for viability percentage among the different doses of Mebendazole, $\chi2(2) = 19.636$, p = 0.003, with mean % viability was 98.47 ± 0.175with dose 0 that was decreases to 55.53 ± 4.326with dose 6 with average percentage decrease was about -43.474. As depicted in figure 3.

Table 1: Comparison of effects of different doses of Mebendazole on MCF-7 cell line viability assess by MTT assay

Doses (µM)	N = 28	Variables				
		Ab'	At	Ac	%	Fa
0	4	3.7±0.5 (3.0-4.3)	0.26 ± 0.01 (0.25 - 0.28)	0.26 ±0.01 (0.25 -0.28)	99.7 ±0.2 (99.4 -100)	0.003 ±0.023 (0 -0.055)
2.5	4	3.7±0.9 (2.5-4.7)	0.24 ± 0.014 (0.23 - 0.26)	0.26 ±0.013 (0.26 -0.28)	89.63 ±0.7 (89.12 -90.72)	0.104 ±0.007 (0.09 -0.11)
5	4	4.2±1.2 (2.5-5.5)	0.20 ± 0.016 (0.19 - 0.23)	0.26 ±0.014 (0.25 -0.28)	76.3 ±2.1 (74.6-79.0)	0.24 ±0.02 (0.21 -0.25)
6	4	4.6±1.0 (3.2-5.5)	0.17 ± 0.012 (0.18 - 0.19)	0.26 ±0.013 (0.25 -0.28)	65.73 ±2.2 (63.55 -67.70)	0.34 ±0.02 (0.32 -0.36)
8	4	3.7±0.82 (2.5-4.2)	0.14 ± 0.014 (0.13 - 0.16)	0.26 ±0.013 (0.25 -0.28)	53.76 ±3.5 (50.2 -57.67)	0.46±0.034 (0.42-0.50)
10	4	4.1±0.83 (3.0-4.8)	0.11 ± 0.019 (0.10 - 0.14)	0.26 ±0.013 (0.25 -0.28)	42.35 ±5.9 (36.9 -48.9)	0.58 ±0.06 (0.51 -0.63)
15	4	4.5±0.35 (4.0-4.75)	0.083 ± 0.02 (0.07 - 0.11)	0.26 ±0.013 (0.25 -0.28)	30.67 ±5.8 (25.97 -38.32)	0.69 ±0.06 (0.62 -0.74)
P-value		0.635	<0.001**	0.824	<0.001**	<0.001**

'Mean ± SD in x10-3
'(Min - Max) in x10-3
**Significant at1%

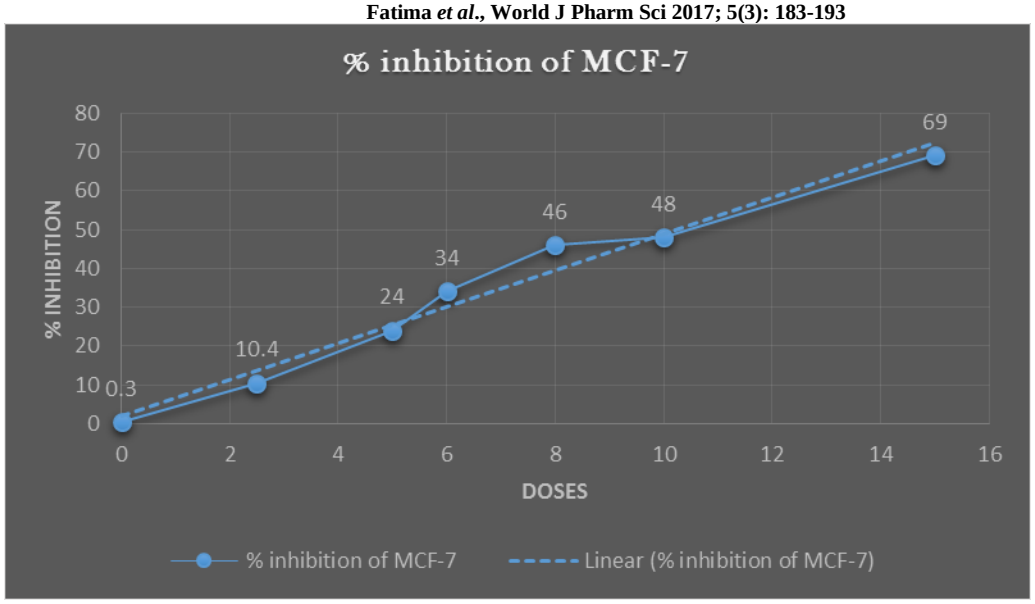

Figure 1: comparison of different doses of Mebendazole on % inhibition of MCF-7 cell line

Table 2: Comparison of effects of different doses of Mebendazole on MDA-MB-231cell line viability assess by MTT assay

Doses (µM)	**N = 28**	**Variables**				
		Ab'	**At**	**Ac**	**%**	**FA**
0	4	3.9 ±0.7	0.34 ±0.023	0.33 ±0.024	99.83 ±0.21	0.002 ± 0.0007
		(3.0 -4.7)	(0.30 -0.35)	(0.30 -0.35)	(99.52 -100)	(0.0017- 0.003)
5	4	4.3 ±0.8	0.31 ±0.023	0.34 ±0.023	92.37 ±0.41	0.08 ±0.004
		(3.5 -5)	(0.28 -0.33)	(0.30 -0.35)	(91.82 -92.75)	(0.07 -0.08)
6	4	4.4 ±0.5	0.28 ±0.026	0.33 ±0.023	83.75 ±1.9	0.16 ±0.019
		(3.7 -5.0)	(0.24 -0.3)	(0.30 -0.35)	(81.25 -85.82)	(0.14 -0.19)
7	4	4.5 ±0.9	0.25 ±0.027	0.33 ±0.023	75.36 ±3.1	0.25 ±0.031
		(3.7 -5.5)	(0.21 -0.28)	(0.30 -0.35)	(70.92 -78.08)	(0.22 -0.29)
9	4	4.6 ±0.5	0.23 ±0.024	0.33 ±0.024	67.1 ±2.7	0.33 ±0.027
		(4.0 -5)	(0.19 -0.25)	(0.30 -0.35)	(63.5 -70.02)	(0.30 -0.36)
12	4	4.2 ±0.9	0.20 ±0.028	0.33 ±0.024	58.34 ±4.4	0.42 ±0.044
		(3.2 -5.5)	(0.16 -0.22)	(0.30 -0.35)	(52.8 -63.5)	(0.376-0.47)
17	4	3.9 ±0.1	0.17 ±0.024	0.33 ±0.026	49.07 ±4.38	0.51 ±0.04
		(2.7 -5.0)	(0.14 -0.19)	(0.30 -0.35)	(44.9-54.35)	(0.45 -0.55)
P-value		0.885	0.001**	0.971	<0.001**	<0.001**

'Mean ± SD in x10-3
'(Min - Max) in x10-3
**Significant at1%

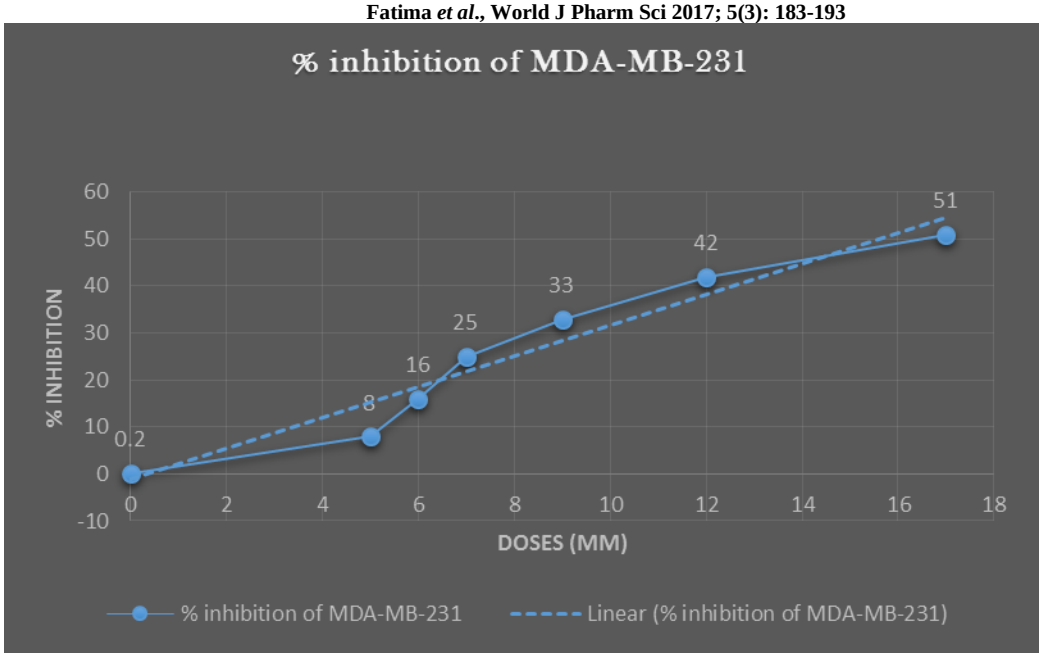

Figure 2: comparison of different doses of Mebendazole on % inhibition of MDA-MB-231 cell line

Table 03: Comparison of effects of different doses of Mebendazole on HT-29 human colorectal adenocarcinoma cell line viability assess by MTT assay

Doses (µM)	**N = 28**	**Variables**				
		Ab'	**At**	**Ac**	**%**	**FA**
0	4	4.0 ±0.7	0.33 ±0.036	0.33 ±0.036	99.76 ±0.20	0.002 ±0.002
		(3.2 -5.0)	(0.30-0.38)	(0.30 -0.38)	(99.57 -100)	(0 -0.004)
2	4	4.0 ±0.1	0.31 ±0.039	0.33 ±0.036	93.13 ±1.5	0.07 ±0.015
		(2.7 -5.5)	(0.28 -0.36)	(0.30 -0.38)	(95.27 -92.0)	(0.05 -0.08)
2.5	4	4.1 ±0.4	0.28 ±0.038	0.33 ±0.036	85.47±2.27	0.14 ±0.023
		(3.7 -4.5)	(0.25 -0.34)	(0.30 -0.38)	(82.97 -88.4)	(0.11 -0.17)
3	4	4.0 ±0.4	0.26 ±0.040	0.33 ±0.036	78.39 ±3.4	0.22 ±0.033
		(3.5 -4.5)	(0.23 -0.31)	(0.30 -0.38)	(74.27 -82.42)	(0.17 -0.26)
4	4	4.0 ±0.5	0.23±0.041	0.33 ±0.037	70.10 ±5.16	0.30 ±0.051
		(3.5 -4.7)	(0.19 -0.29)	(0.30 -0.38)	(63.4 -75.7)	(0.24 -0.36)
5	4	4.1 ±0.1	0.21 ±0.043	0.33 ±0.036	62.8 ±5.9	0.372 ±0.059
		(4.0 -4.2)	(0.17 -0.27)	(0.30 -0.38)	(56.77 -70.77)	(0.29 -0.43)
5.5	4	3.9 ±0.43	0.183 ±0.046	0.324 ±0.036	55.11 ±7.87	0.45 ±0.079
		(3.5 -4.25)	(0.14 -0.25)	(0.30 -0.38)	(46.17 -65.27)	(0.35 -0.54)
P-value		0.983	0.005**	0.947	<0.001**	<0.001**

'Mean ± SD in x10-3
'(Min - Max) in x10-3
**Significant at1%

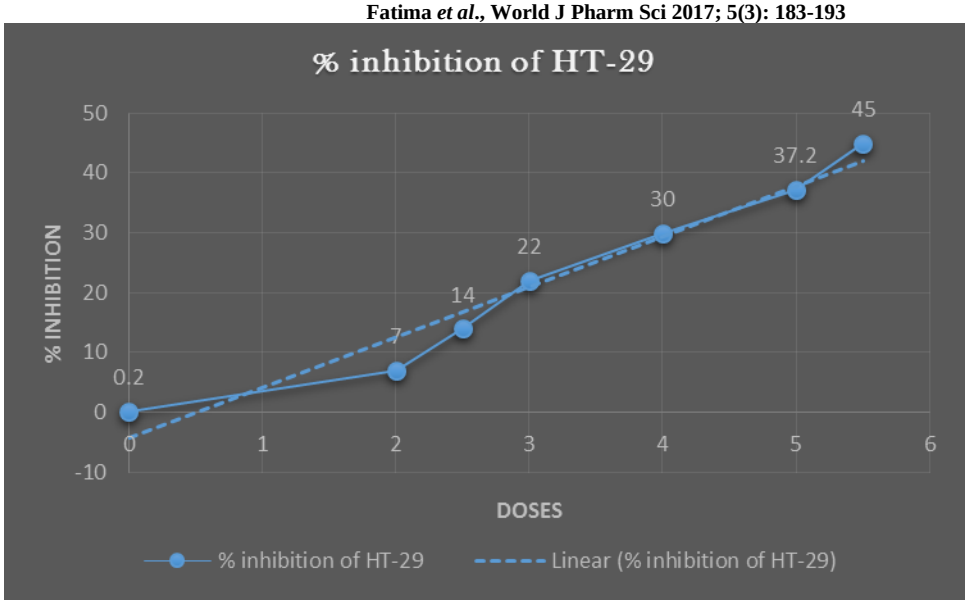

Figure 3: comparison of different doses of Mebendazole on % inhibition of HT-29 cell line

Table 4: Comparison of effects of different doses of Mebendazole on Hela cell line viability assess by MTT assay

Doses(µM)	**N = 28**	**Variables**				
		Ab'	**At**	**Ac**	**%**	**FA**
0	4	3.75 ±0.5	0.393 ±0.014	0.394 ±0.014	99.79 ±0.103	0.021 ±0.001
		(3.0 –4.2)	(0.38 -0.41)	(0.38 -0.41)	(99.67 –99.92)	(0.008 -0.033)
5	4	3.94 ±0.4	0.37 ±0.015	0.393 ±0.014	93.82 ±0.64	0.061 ±0.006
		(3.5 –4.2)	(0.35 -0.39)	(0.38 -0.41)	(93.22 -94.7)	(0.053 -0.067)
10	4	4.25 ±0.61	0.35 ±0.016	0.393 ±0.015	87.856 ±1.2	0.121 ±0.012
		(3.5 -5.0)	(0.332 -0.361)	(0.38 -0.41)	(86.2 -88.82)	(0.112 -0.14)
12	4	4 ±0.43	0.323 ±0.015	0.392 ±0.015	81.78 ±1.1	0.182 ±0.011
		(3.5 –4.5)	(0.308 -0.34)	(0.377 -0.406)	(81.0 -83.22)	(0.17 -0.20)
15	4	3.4 ±0.9	0.30 ±0.014	0.392 ±0.014	75.63 ±1.43	0.244 ±0.014
		(2.2 -4.5)	(0.285 -0.312)	(0.38 -0.41)	(73.88 -77.35)	(0.23 -0.261)
20	4	3.12 ±0.6	0.275 ±0.012	0.391 ±0.015	69.6 ±0.95	0.304 ±0.094
		(2.2-3.7)	(0.262 -0.285)	(0.38 -0.41)	(68.62 -70.82)	(0.291 -0.314)
25	4	3.7 ±0.55	0.25 ±0.012	0.39 ±0.015	63.144 ±0.90	0.37 ±0.09
		(3.2 -4.5)	(0.24 -0.262)	(0.376 -0.405)	(61.95 -63.9)	(0.361-0.38)
P-		0.305	<0.001**	0.972	<0.001**	<0.001**

'Mean ± SD in x10-3
'(Min - Max) in x10-3
**Significant at1%

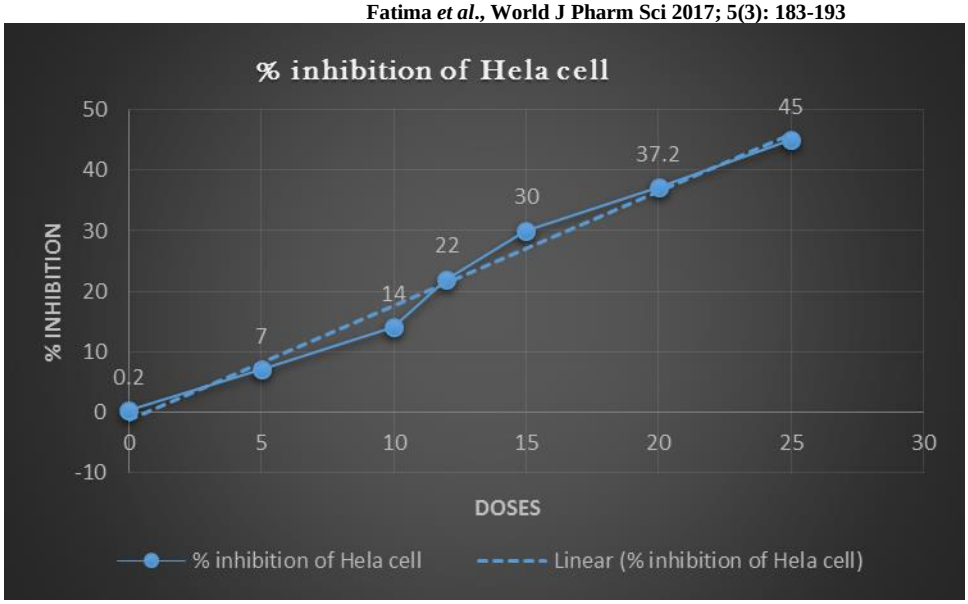

Figure 4: comparison of different doses of Mebendazole on % inhibition of Hela cell line

Table 05: Comparison of effects of different doses of Mebendazole on MCF-10 cell line viability assess by MTT assay

Doses (µM)	N = 28	Variables				
		Ab'	At	Ac	%	FA
0	4	3.8 ± 0.4	0.481 ± 0.008	0.482 ± 0.008	99.76 ± 0.086	0.002 ± 0.0008
		(3.5 - 4.2)	(0.47 - 0.49)	(0.471 - 0.49)	(99.65 - 99.85)	(0.0015 - 0.0035)
35	4	4.4 ± 0.5	0.481 ± 0.008	0.48 ± 0.008	99.66 ± 0.18	0.003 ± 0.0018
		(3.7 – 4.7)	(0.471- 0.49)	(0.47 - 0.49)	(99.42 - 99.82)	(0.002 - 0.006)
65	4	4.5 ± 0.5	0.48 ± 0.008	0.48 ± 0.008	99.48 ± 0.422	0.005 ± 0.004
		(4.0 – 5.2)	(0.47 - 0.48)	(0.467- 0.49)	(99.00 – 100.0)	(0.000- 0.01)
75	4	4.7 ± 0.3	0.474 ± 0.007	0.477 ± 0.007	99.31 ± 0.382	0.007 ± 0.004
		(4.2- 5)	(0.465 - 0.483)	(0.466 - 0.484)	(98.75 - 99.60)	(0.004 - 0.012)
85	4	4.8 ± 0.4	0.473 ± 0.008	0.475 ± 0.007	99.18 ± 0.683	0.008 ± 0.007
		(4.2- 5.0)	(0.465 - 0.482)	(0.465 - 0.482)	(98.22 - 99.8)	(0.002 - 0.018)
95	4	4.5 ± 0.3	0.47 ± 0.0084	0.474 ± 0.008	98.90 ± 1.03	0.011 ± 0.0102
		(4.2 - 5.0)	(0.462 - 0.48)	(0.462 - 0.48)	(97.42 – 99.65)	(0.0035 - 0.026)
110	4	4.2 ± 0.5	0.47 ± 0.009	0.473 ± 0.008	98.72 ± 0.973	0.012 ± 0.009
		(3.7 - 5.0)	(0.46 - 0.48)	(0.4763- 0.48)	(97.55 - 99.52)	(0.005 - 0.0245)
P-		0.106	0.174	0.453	0.109	0.109

'Mean ± SD in x10-3; '(Min - Max) in x10-3

Table 6: Comparison of IC50 values of Mebendazole among all treated cells

Cell Types (N=4)	Mean±SD	P-Value
MCF-7	7.449 ± 0.535; (7.6045- 8.235)	0.002**
MDA-MB-231	7.68± 0.442; (7.16 - 8.23)	
HT-29 human colorectal adenocarcinoma	2.59 ± 0.218; (2.34 - 2.87)	
Hela cell line	22.36 ± 4.315; (18.56 - 27.19)	
MCF-10	849.12 ± 23.96; (827.61 - 873.08)	

Mean ± SD; (Min - Max); **Significant at 1%

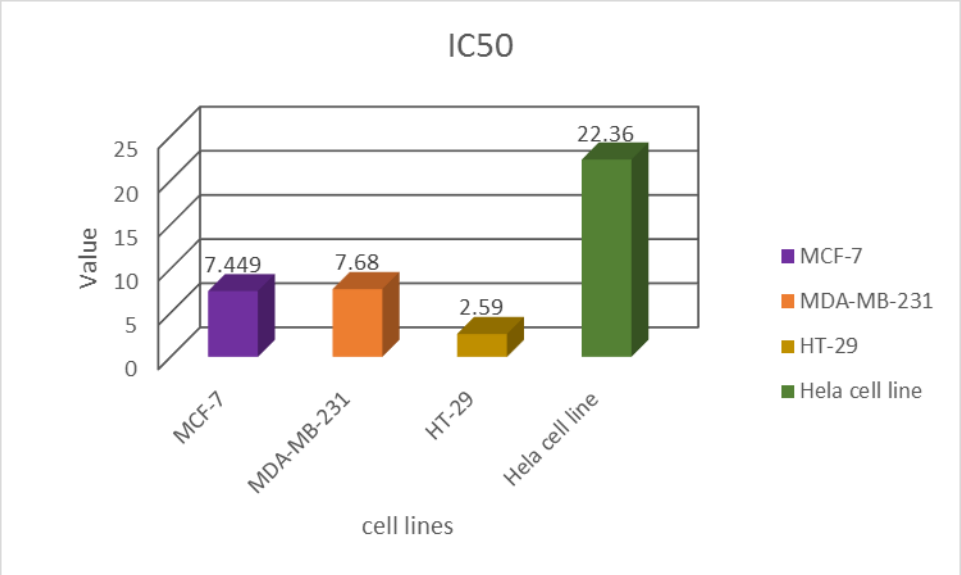

Figure 5: Comparison of IC50 values of Mebendazole among study cancerous cell lines

Table 07: Comparison of Selective Index of Mebendazole among MCF-7 and MDA-MB-231 cell lines

Cells Type	N	SI
MCF-7	16	114.865 ± 11.20
		(87.883 - 132.027)
MDA-MB-231	16	111.245 ± 8.99
		(89.88 - 132.027)
P-value		0.291

(Min - Max); Mean ± SD

Table 08: Comparison of effects of different doses of Mebendazole on MCF-7 evaluate by Trypan blue dye exclusion assay

Doses (µM)	N	Viable Cells	Total Cells	Viability (%)	Death cells
0	3	261.92 ± 1.182	267.7 ± 1.226	98.47 ± 0.175	4.852±0.0448
		(260.6 – 263.0)	(265.4 – 267.9)	(98.34 - 98.67)	(4.8-4.88)
5	3	248.3 ± 3.704	266.1 ± 1.312	92.49 ± 0.232	17.818±3.24
		(244.9 – 252.3)	(264.7 – 267.3)	(92.22 - 92.65)	(14.08-19.825)
10	3	231.86 ± 5.071	265.6 ± 1.246	86.43 ± 0.703	33.765±4.72
		(227.6 – 237.4)	(264.3 – 266.7)	(85.625 - 86.86)	(28.32-36.675)
12	3	213.6 ± 5.370	264.9 ± 1.166	79.86 ± 0.834	51.33±5.107
		(209.65 – 219.7)	(263.67 – 265.97)	(78.92 - 80.52)	(45.44-54.525)
15	3	196.16 ± 7.325	264.35 ± 1.099	73.13 ± 1.197	68.195±7.142
		(191.2 – 204.5)	(263.2 – 265.3)	(71.80 - 74.12)	(59.96-72.70)
20	3	178.9 ± 1.184	263.7 ± 1.103	66.34 ± 1.688	84.862±11.545
		(170.3 – 1.924)	(262.5 – 264.7)	(64.52 - 67.86)	(71.56-92.275)
25	3	160.33 ± 1.444	263.24± 1.029	55.53 ± 4.326	102.915±14.148
		(105.005 – 176.8)	(262.1 – 264.15)	(50.62 - 58.78)	(86.62-112.075)
P-value		0.004**	0.049*	0.003**	0.004**

'Mean ± SD in x 10^4: '(Min - Max) in x 10^4: **Significant at 1% ; *Significant at 5%

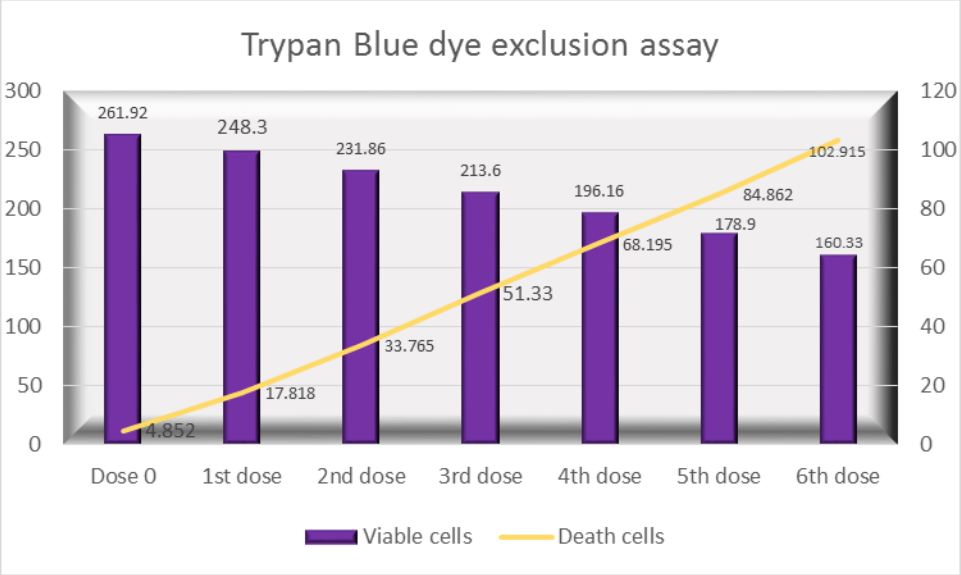

Figure 6: Comparison of different doses of Mebendazole on Viable and Death cells count of MCF-7 assessed by Trypan Blue dye exclusion assay

DISCUSSION

Mebendazole is well known efficacious antiparasitic agent that nowadays gain importance because of its potential anti-tumor activity beside its primary activity against parasites. A preventive effect of Mebendazole on colonic carcinomas was the main reason that brought this agent in limelight for oncology researchers as an anti-tumor agent[xv]. But unfortunately the beneficial anti-tumor effects of Mebendazole on breast cancer cell lines were not fully established. For this reason, in this research project, we were evaluating this important aspect by using different cancerous cell lines. Cancer cell lines model was generally utilized for anti-cancerous drugs evaluation for advancement of treatment approach against cancer. Cytotoxicity against these cell lines can be assessed by different assays to assess viable cells by either membrane integrity (dye exclusion assay most importantly trypan blue dye exclusion assay), mitochondrial enzymatic activity (most commonly by MTT assay) and nuclear activity (most commonly by TUNEL assay)[xvi]. For assessment of in-vitro cytotoxicity of Mebendazole, we used MTT assay for all cell lines and trypan blue dye exclusion assay for only MCF-7.

For MCF-7 after 72 hours of incubations with different dilutions of Mebendazole leads to significant reduction of absorbance values of At with mean percentage decrease was about -68.972 ± 5.557 from dose 0 to dose 6th of Mebendazole alone along with significant reduction of %viability of MCF-7 from 99.69 ± 0.2 to 30.68 ± 5.8 as assessed by MTT assay. This was matched with the study conducted by Hou et al. (2015)[xvii]. As they demonstrating the anti-cancerous activity of derivative of Benzimidazole, Flubendazole on MCF-7, MDA-MB-231, BT-549 and SK-BR-3 cell lines. They revealed that Flubendazole significantly decreases the proliferation of these cell lines with mean IC50 values were 5.51 ± 1.28, 1.75 ± 1.27 for MCF-7 and MDA-MB-231 cell lines. As cytotoxicity assay for MCF-7 cell line assessed by trypan blue dye exclusion assay revealed that Mebendazole significantly decreases the viable cells counts from 261.92 ± 1.182 to 160.33 ± 1.444 along with significant reduction of % viability. As observed by Zhou et al. (2014) that Mebendazole able to decreasing the viability of cancerous cells through prompting cellular apoptosis by phosphorylating antiapoptotic protein (Bcl-2) sequentially increasing the caspase activity[xviii]. Additionally, Zanganeh et al.(2016)[xix]demonstrated that Mebendazole can decrease the viability of breast cancerous cells by means of increasing the apoptosis of cancerous cells by increasing the release of cytochrome c from mitochondria into cytoplasm which in turn initiating apoptosis process by binding with Apoptotic protease activating factor 1, (APAF1).

For MDA-MB-231 cell Mebendazole significantly decreases the percentage viability from 99.83 ±0.21 to 49.07 ±4.38 in dose dependent manner with mean IC50 value was 7.68± 0.442. This showed that Mebendazole disrupting the polymerization of tubulin and arresting the cellular growth at G2/M phase and ultimately causing apoptosis of cancerous cell lines, these finding also observed by Sawanyawisuth et al. (2014). As they demonstrated

that Mebendazole suppress the tumor activity of cholangiocarcinoma cell line effectively. Another study performed by Pinto et al.(2015)[xx]explained that Mebendazole is significantly effective in inhibiting the cellular proliferation, invasion and migration of gastric cancer cell lines.

Correspondingly for HT-29 cell line Mebendazole can commendably decreases the percentage viability with mean value was 99.76 ±0.20 with dose 0 that decreases to 55.11 ±7.87 with maximum dose of Mebendazole and mean IC50 value was 2.59 ± 0.218. These findings were in line with the study conducted by Nygren et al.(2013)[xxi]. They evaluated the anticancerous activities of Mebendazole and Albendazole on several colonic cancer cell lines including HCT 116,RKO, HT29, HT-8 and SW626 and the non-malignant epithelial cell lines including MCF 10A, RPTEC/TERT1 and NeHepLxHT cell lines that representing the normal breast epithelial, renal and hepatocytes respectively. They concluded that Mebendazole as compare to Albendazole more efficiently and selectively decreases the proliferative activities of colonic cancer cell lines with mean IC50 were less than 5mM and relatively inactive in other normal epithelial cell lines.

Mebendazole exhibited statistically non-significant changes of percentage viability of MCF-10 cell line with mean % viability was 99.76 ± 0.086 for dose 0 that decreases to 98.72 ± 0.973 for dose 6. This showed that Mebendazole having more selectivity towards cancerous cells because it can't be able to inhibit the normal endothelial cell growth by efficiently and inhibiting the neovascularization of tumor cells and starving them without affecting the normal epithelial cells[vii]. As cancer treatment is still challenging in terms of effectiveness, economical burden and safety so addition of newer and safer economical option that is also having selective cytotoxicity against cancerous cells will be valuable option for chemotherapy. Hence, Mebendazole may be a valuable option for cancer treatment.

CONCLUSION

This study demonstrates that Mebendazole is effective in decreasing the percentage viability of breast cancerous cell lines MCF-7, MDA-MB-231 with little or no effects on MCF-10 in-vitro. This shows that Mebendazole would be a valuable addition in chemotherapeutic field in terms of selectively inhibiting the cancerous cells.

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