Figure 1: The percentage of viable cells in comparison to untreated cells following 5 hours of incubation. The data was the average of measurement from three different set of experiments. Single tailed error bar was the +3 S.E.M.

Based on a study, cells treatment for 5 hours did not show any sign of immediate cell death (Figure 1). This may indicate that the aqueous extracts of NS did not kills the MCF-7 immediately and some molecular events may be involved [13]. Although not significant, it is worth to emphasize that the concentration below 31.25 µg/ml did not cause any changes compared to untreated cells (medium only), but slight changes occurs in the cell viability if the concentration is over 62.5 µg/ml. In contrast to 5 hours, both 24 hours and 48 hours of incubation with NS extract shows significant changes in the cell viability in a pattern of proportional to the concentration (Figure 2 and Figure 3).

Figure 2: The percentage of viable cells in comparison to untreated cells following 24 hours of incubation. The data was the average of measurement from three different set of experiments. Single tailed error bar was the +3 S.E.M.

Figure 3: The percentage of viable cells in comparison to untreated cells following 48 hours of incubation. The data was the average of measurement from three different set of experiments. Based on a research, single tailed error bar was the +3 S.E.M. In comparison of 24 hours incubation, 48 hours incubation with aqueous extracts of NS shows more significant inhibition. For 48 hours incubation, the lowest concentration of extract shows inhibition near to 50% of MCF-7 population. It is in line with the doubling time for MCF-7 is every 24 hours [13,14].

Figure 4: The percentage of viable cells in comparison to untreated cells following 5, 24 and 48 hours of incubation. The data was the average of measurement from three different set of experiments. Dotted line indicates the concentration equivalent of 50% of viable cells. Error bar was the +3 S.E.M. The optimal concentration was determined by using IC50 plot method. The percentage of viable cells were plotted in a line chart and the inhibition to 50% of the MCF-7 population was extrapolated. Figure 4 clearly shows that the IC50 for both 24 hours and 48 hours treatments are at the almost similar range of concentration. The IC50 for both falls between 11.7 to 12 µg/ml.

3.3 Morphological changes upon treatment with Nigella sativa

The morphological changes of MCF-7 breast cancer cell in response to Nigella sativa extract was evaluated quantitatively and qualitatively. The area of plasma membrane and nucleus of the cells was used to measure the ratio of the area. The mean and standard deviation of the ratio area were used to get the overall result of the treatments. All the calculated data of all treatment and controls are tabulated in Table 2.

Table 2: Mean and standard deviation of ratio area

Treatments	5 Hours	24 Hours	48 Hurs
Q5 medium	4.97 ±1.46	2.24 ±0.55	None
PBS	3.30 ±1.38	2.04 ±0.32	None
500 µg/ml *N.sativa*	None	None	None
15.63 µg/ml *N.sativa*	2.62 ±0.58	None	None
0.97 µg/ml *N.sativa*	2.22 ±1.07	None	None

The mean of ratio calculated showed that the size of the cells treated after hours is larger than 24 hours for the respective treatment. There is no calculated ratio obtained for all Nigella sativa treatments induced after 24 hours exposure. Nearly double the size of reduction from the positive control for the 15.63 µg/ml and 0.97 µg/ml of extracts in 5 hours treatment.

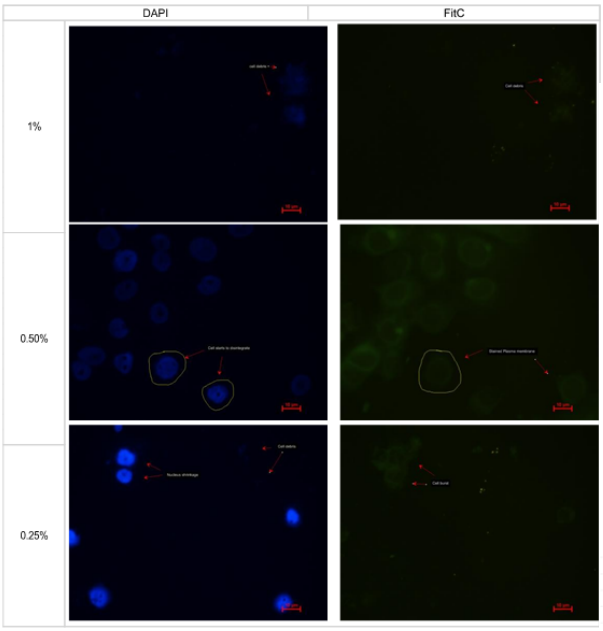

Figure 5: Morphology of the cells for 500, 15.63 and 0.97 µg/ml treatments after 5 hours exposure

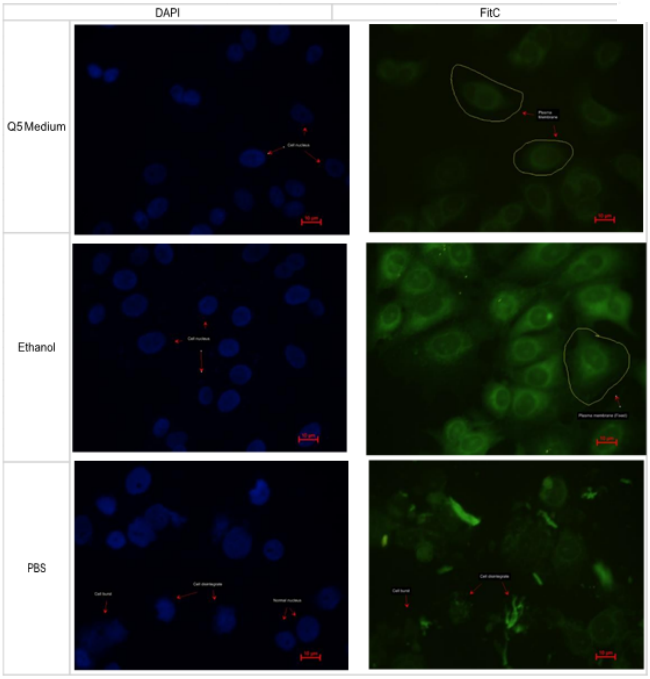

Figure 6: Morphology of the cells for all controls treatment after 5 hours exposure

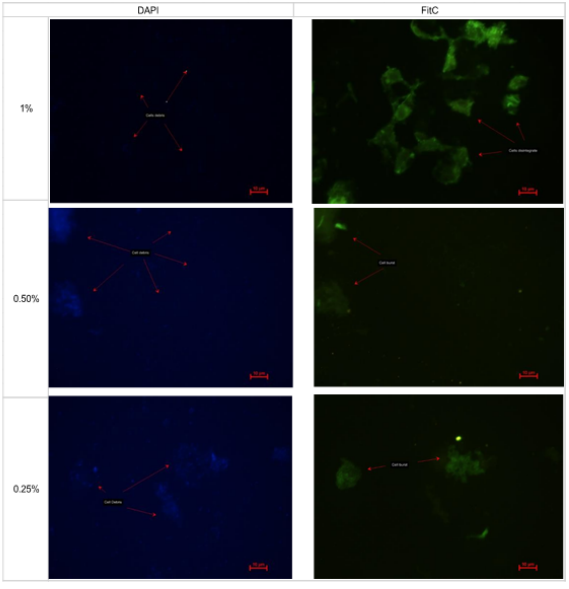

Figure 7: Morphology of the cells for 500, 15.63 and 0.97 µg/ml treatment after 24 hours exposure

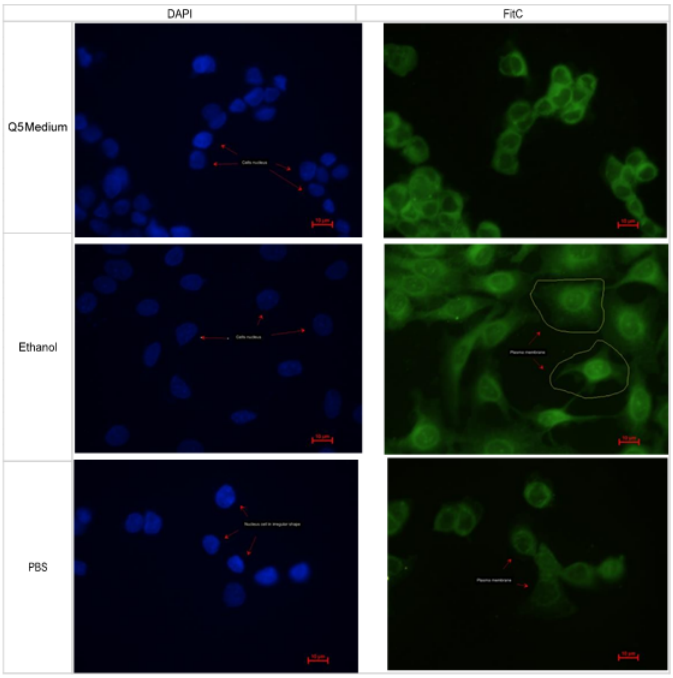

Figure 8: Morphology of the cells for all controls treatment after 24 hours exposure

Figure 5 to 8 shows the microscopy appearance of MCF-7 after the treatment with selected concentration of NS extract for 5 and 24 hours. No microscopy images were recorded for 48 hours of treatment due to the inability to locate live cells. Figure 5 and 7clearly indicates that the integrity of the cells were damaged following 24 hours and 48 hours of treatment with any concentration of NS extracts. In highlight, NS extracts was found to increase the average size of nucleus with slight fragmentation and decreasing the average size of MCF-7 cytoplasm which could be the indicator for cell death mechanism. Based on a study, NS extract was found to induce program cell death through caspase 3, 8, 9 and BAX pathway [15].

Interestingly, in all cell viability experiment the viability of MCF-7 treated with ethanol were reduced to near 1%, but microscopy images show an intact MCF-7. Research shows that this may due to the nature of ethanol that can fix the cells on the glass slide, thus keep the morphology intact although no metabolic activity as showed by MTT assay [4].

In general, aqueous extract of NS shows significant inhibition in the viability of MCF-7 in the pattern of concentration and incubation period dependent. Although the use of MTT has been used in many viability studies, it was recommended to include some microscopy analysis as it can supply the information of the cells integrity [16].

4. CONCLUSION

Aqueous extracts of Nigella sativa have water soluble active compound that can inhibit the growth and viability of MCF-7 in incubation period and concentration dependent relationship. The extract also can reduce the integrity of MCF-7 cell membrane and causing the swelling of its nucleus.

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