exposed by 200 μM of curcumin. Group V : with exposed by 400 μM of curcumin, Group VI : with exposed by 800 μM of curcumin. Each group replicate five times, and each will be the nexamined by two kind of examination, proliferation and apoptosis index.

Proliferation Index

Proliferation index was measured by calorimetric examination which is called the method of MTT proliferation index. This method measuring absorbance of formazan, which it's produce by proliferative cells. The higher the absorbance value means more formazan produced, and indicates a growing number of proliferating cells.

Apoptosis Index

Apoptosis index examination by the method of labeling DNA fragmentation TUNEL system. Cell undergoing apoptosis was characterized by brown staining in the nucleus.

Ethics

Ethical clearance was obtained from Health Research Ethics Committee of Dr. Saiful Anwar General Hospital.

Statistical Analysis

Data are analyzed using one-way ANOVA test and followed by Least Significant Difference (LSD) to differences between groups. Post hoc test was used if the ANOVA was significant. P < 0.05 was considered statistically significant.

RESULTS

Culture of Complete Hydatidiform Mole

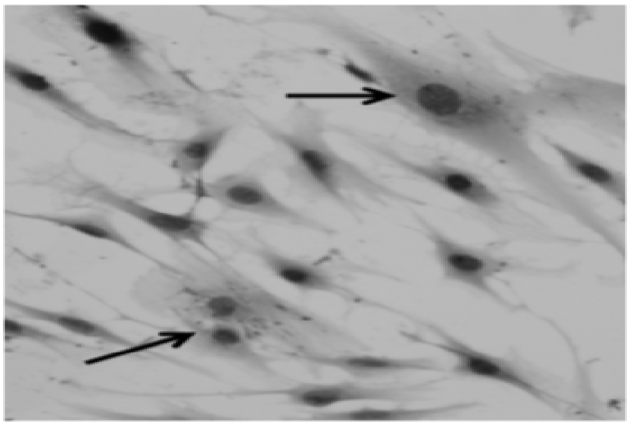

Figure 1. Trophoblast Cell Culture of Complete Hydatidiform Mole.

Proliferation Index Analysis

Proliferation index in CHM trophoblastic cells can be seen in Table 1 and Fig. 1. Table 1 shows mean data of proliferation index according to varrying doses of curcumin.

Table 1. Mean Data of Proliferation Index According to Varrying Doses of Curcumin

Treatment	Proliferation index Means ± SD	p-value
Control	0.96 ± 0.20^{a}	
Curcumin 50 μM	1.10 ± 0.10^{a}	
Curcumin 100 μM	0.64 ± 0.37^{a}	
Curcumin 200 μM	0.36 ± 0.03^{c}	0.001
Curcumin 400 μM	0.34 ± 0.02^{c}	
Curcumin 800 μM	0.32 ± 0.01^{c}	

Description : at the mean ± SD, if it contains different letters mean no significant difference (p-value <0.05) and if it contains the same letters mean no significant difference (p-value>0.05).

The table shows that treatment of varying doses of curcumin have different influence on mean proliferation index. ANOVA test showed significant differences (p<0,001) in proliferation index in CHM trophoblastic cells with various doses of curcumin treatment and control.

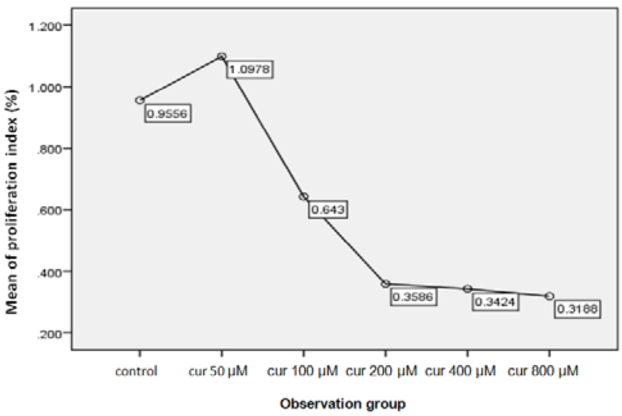

Figure 2. Trend Change in Mean Proliferation Index

Apoptosis Index Analysis

Description : cells undergoing apoptosis are characterized by brown-staining in the nucleus (marked with circles) and cells which not undergoing apoptosis showed no brown staining in the cell nucleus (marked with a circle cut off).

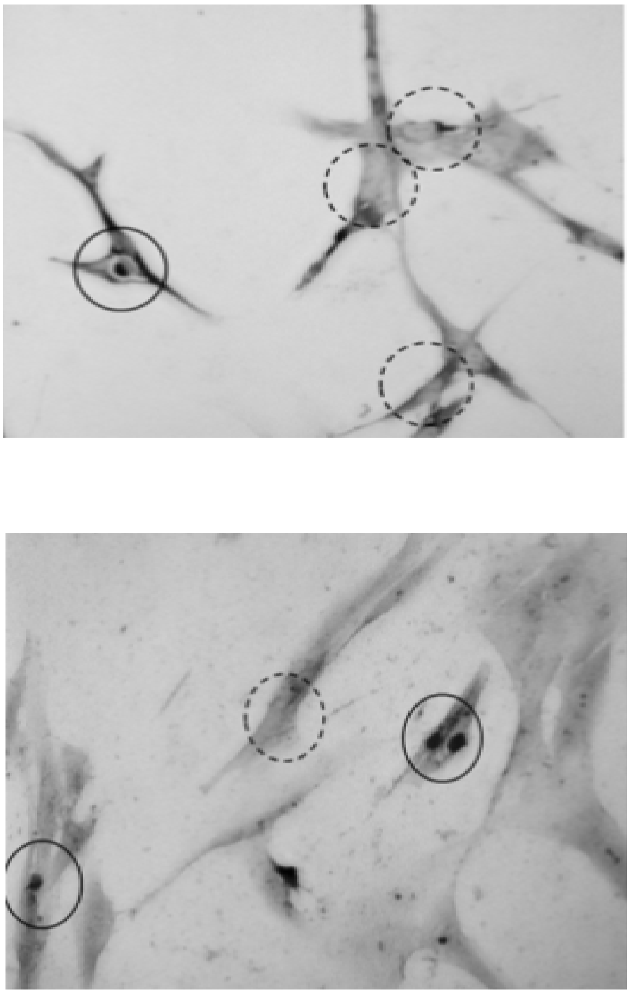

Figure 3. Immunohistochemical TUNEL Labeling System Introphoblast Cells CHM with 400x Magnification.

Table 2. Mean Data of Apoptosis Index According to Varying Doses of Curcumin

Treatment	Apoptosis index Means ± SD	p-value
Control	14.4 ± 4.56^{a}	
Curcumin 50 μM	18.8 ± 5.02^{a}	
Curcumin 100 μM	22.4 ± 7.13^{a}	0.001
Curcumin 200 μM	44.4 ± 7.40^{b}	
Curcumin 400 μM	56.8 ± 13.16^{b}	
Curcumin 800 μM	49.6 ± 16.15^{b}	

Description: at the mean ± SD, if it contains different letters mean no significant difference (p-value <0.05) and if it contains the same letters mean no significant difference (p-value>0.05).

This table shows that treatment of varying doses of curcumin have different influence on mean apoptosis index. ANOVA test showed significant differences (p<0,001) in apoptosis index in CHM trophoblastic cells with various doses of curcumin treatment and control.

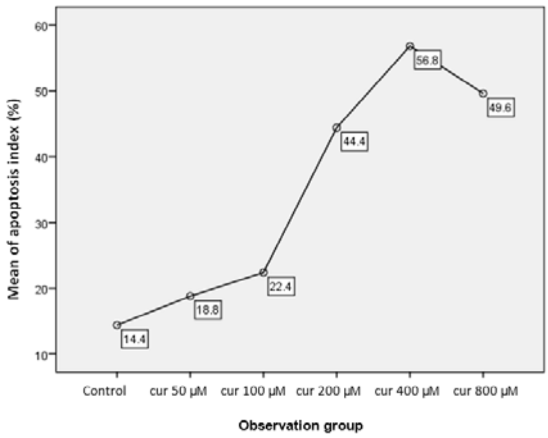

Figure 4. Trend change in mean apoptosis index.

DISCUSSION

Curcumin is a compound derivative of turmeric which proved capable of inhibiting tumor transformation, initiation and promotion, proliferation, invasion, angiogenesis and metastasis. Many studies have shown that curcumin modulates a variety of molecular targets, including growth factors and their receptors, transcription factors, cytokines, enzymes and genes that regulate apoptosis. Curcumin inhibits proliferation of cancer cells by holding them at different stages of the cell cycle and inducing apoptosis.[10-12]

Curcumin proved to inhibit cell proliferation, namely through inhibition of NFkB, in several types of cancer by lowering protein antiapoptosis (Bcl-2 and Bcl-XL), cell cycle regulators (cyclin-cyclin D-1 and D-2), growth factors (interleukin, TNF-α, VEGF) and increase apoptosis, by activating caspase.[10-12]

In this study, the one-way ANOVA test proved there were not significant differences in decreasing proliferation index between control group and curcumin dose 50 μM. And there were highly significant differences (p=0.001) indecreasing of proliferation index by curcumin administration of a dose of 100 μM, 200 μM, 400 μM and 800 μM compared with the control and curcumin dose of 50 μM (Table 1). Figure 1 shown that proliferation index decreased with increasing doses of curcumin. However, there was no significant difference in reduction in proliferation index between the treatment dose administration of curcumin at a dose of 200 μM, 400 μM and 800 μM. So it means that statistically the third dose has the same capabilities in terms of reducing the proliferation index in cell culture of complete hydatidiform mole.

In this study, the one-way ANOVA test obtained a very significant difference in the mean apoptosis index of cells into six groups of sample observations, as shown by the p-value=0.001. With LSD in Table 2 shows that there is no significant difference in the mean apoptosis index of cells between the control group and the group treated with curcumin dose administration of 50 μM and also with a dose of 100 μM. This suggests that there is no effect of giving curcumin 50 μM and 100 μM against cell apoptosis index in cell culture complete hydatidiform mole. Although, it appears to be an increase in the mean value but the increasement was not statistically significant.

From this research shows that there are significant differences in the apoptosis index between the control group and the group given doses of curcumin 200 μM, 400 μM, ora dose of 800 μM. Figure 3 shown that apoptosis index increase with increasing doses of curcumin. However, there was no significant difference in increasing of apoptosis index between the group given doses of curcumin 200 μM, 400 μM, or 800 μM. In other words, the three doses of curcumin have the same ability to increasing the apoptosis cells index in cell culture of complete hydatidiform mole. Where the average value of the cell apoptosis index was highest at a dose of 400 μM. In this study a dose of 400 μM can be considered as the most optimal dose of curcumin in increasing cell apoptosis index.

CONCLUSION

Based on the results and discussion in this study it can be concluded that the administration of curcumin can lowering proliferation index and increasing apoptosis index in trophoblastic cell culture of complete hydatidiform mole. Where dose of curcumin that proved significant in reducing the proliferation index and increasing the apoptosis index in this study is a dose of 200 μM.

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