CHAPTER III

RESULTS

3.1 Cytotoxicity of turmeric curcuminoids on leukemic cell lines

To examine whether exposure of curcuminoid mixture, pure curcumin, demethoxycurcumin, and bisdemethoxycurcumin affects the viability of cells, 4 leukemic cell lines, including human erythriod leukemia (K562), human promyeloid leukemia (HL-60), human monocytic leukemia (U937) and human lymphoblastic leukemia (Molt4), were exposed to various concentrations of curcuminoids (0-136 µM for curcuminoid mixture and pure curcumin, 0-148 µM for demethoxycurcumin, and 0-162 µM for bisdemethoxycurcumin) for 48 h and cell viability was determined by the MTT assay as described in section 2.5. The Curcuminoid mixture showed cytotoxic effects on leukemic cell lines, with the inhibitory concentration at 50% (IC_{50}) approximately 24.2 ± 8.4, 19.1 ± 0.8, 24.1 ± 1.4, and 13.0 ± 0.1 µM in K562, U937, HL-60, and Molt4, respectively (Figure 8). The IC_{50} of pure curcumin were 34.1 ± 10.8, 22.1 ± 1.4, 28.6 ± 5.4, and 13.4 ± 0.1 µM in K562, U937, HL-60, and Molt4, respectively (Figure 9). The IC_{50} of demethoxycurcumin were 32.0 ± 2.6, 24.8 ± 2.4, 26.4 ± 8.8, and 14.0 ± 0.1 µM in K562, U937, HL-60, and Molt4, respectively (Figure 10). The IC_{50} of bisdemethoxycurcumin were 42.0 ± 6.5, 23.2 ± 4.2, 30.5 ± 9.7, and 16.7 ± 0.2 µM in K562, U937, HL-60, and Molt4, respectively (Figure 11). The values of IC_{50} of curcuminoid on leukemic cell lines are summarized in Table 20. In addition, the cytotoxic concentration at 20% (IC_{20}) of curcuminoids on K562, U937, HL-60, and Molt4 ranged from 8.5 ± 0.3 to 14.8 ± 1.4 µM, as shown in Figures 8 to 11 and Table 21. Non-cytotoxic concentrations (IC_{20}) for all curcuminoid derivatives were used for further experiments.

3.2 Level of *WT1* gene expression in leukemic cell lines

To clarify whether the expression levels of *WT1* gene in all leukemic cell lines was sufficient for detection after treatment with curcuminoids, 4 types of untreated leukemic cell lines were grown in complete RPMI 1640 medium. After reaching 80%

cell confluence, total RNA extraction (1 µg/reaction) and RT-PCR were carried out. The PCR products (15 µL) were electrophoresed and visualized using a scan densitometer, as described in sections 2.6 and 2.7. The result showed that the levels of *WT1* gene expression in all leukemic cell lines were detected as shown in Figure 12.

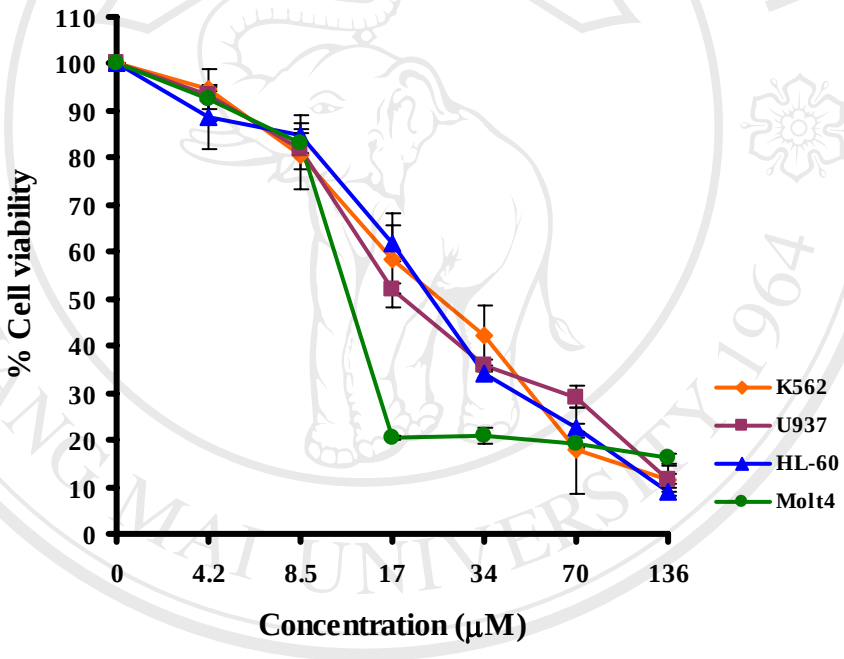

Figure 8. Cytotoxicity of curcuminoid mixture on leukemic cell lines. Leukemic cell lines ($1x10^5$ cells/well) in 200 µL medium were grown in the presence of various concentrations of curcuminoid mixtures for 48 h. The cell viability was determined by MTT assay. Each point represents the mean value ± standard deviation (SD) of three independent experiments performed in triplicate.

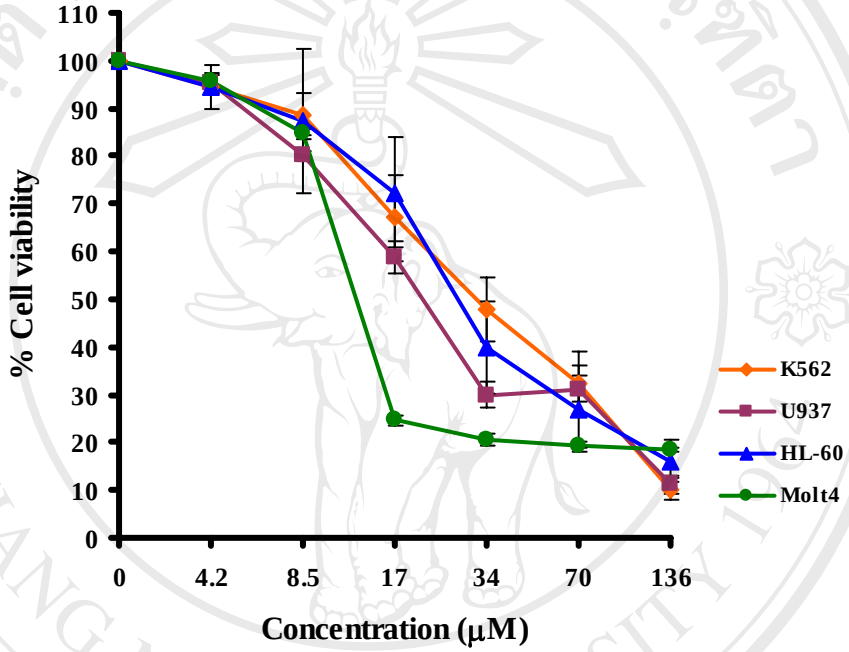

Figure 9. Cytotoxicity of pure curcumin on leukemic cell lines. Leukemic cell lines ($1x10^5$ cells/well) in 200 µL medium were grown in the presence of various concentrations of pure curcumin for 48 h. The cell viability was determined by MTT assay. Each point represents the mean value ± SD of three independent experiments performed in triplicate.

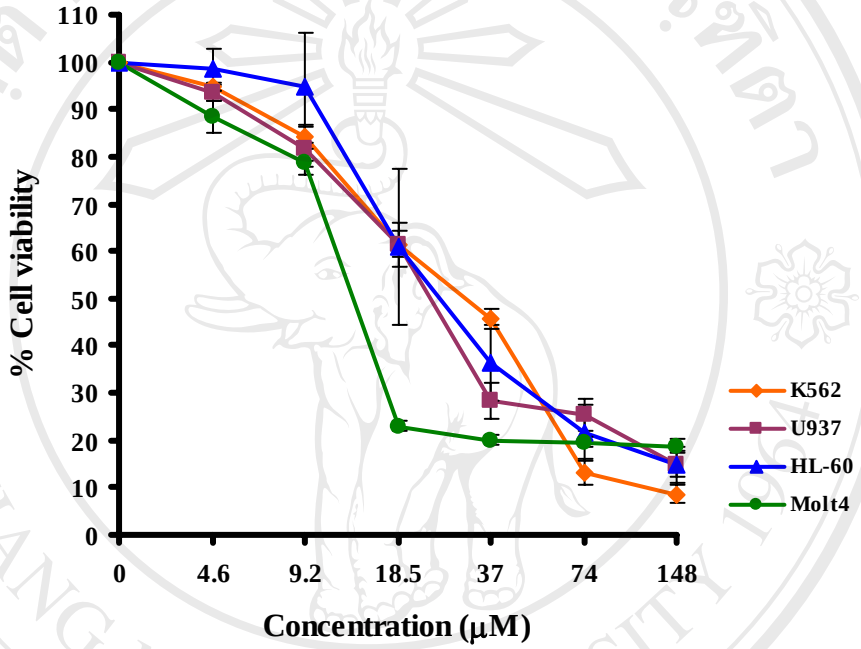

Figure 10. Cytotoxicity of demethoxycurcumin on leukemic cell lines. Leukemic cell lines ($1x10^5$ cells/well) in 200 µL medium were grown in the presence of various concentrations of demethoxycurcumin for 48 h. The cell viability was determined by MTT assay. Each point represents the mean value ± SD of three independent experiments performed in triplicate.

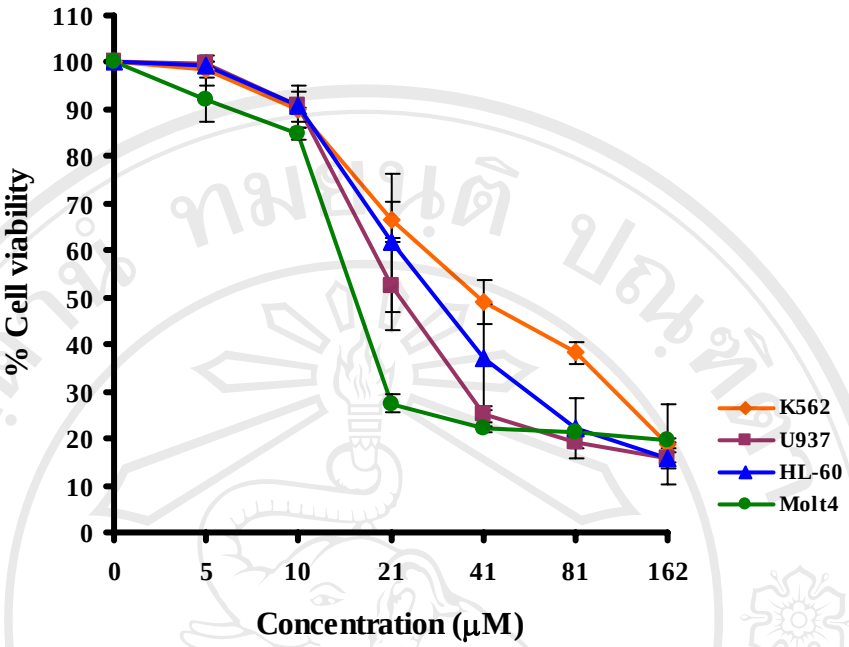

Figure 11. Cytotoxicity of bisdemethoxycurcumin on leukemic cell lines. Leukemic cell lines ($1x10^5$ cells/well) in 200 µL medium were grown in the presence of various concentrations of bisdemethoxycurcumin for 48 h. The cell viability was determined by MTT assay. Each point represents the mean value ± SD of three independent experiments performed in triplicate.

Table 20. IC_{50} values of curcuminoids derivatives on cytotoxicity of leukemic cell lines. The data represent the mean values (µM) ± SD of three independent experiments performed in triplicate.

Turmeric curcuminoids	IC_{50} on leukemic cell lines (µM)			
	K562	U937	HL-60	Molt4
Curcuminoid mixture	24.2 ± 8.4	19.1 ± 0.8	24.1 ± 1.4	13.0 ± 0.1
Pure curcumin	34.1 ± 10.8	22.1 ± 1.4	28.6 ± 5.4	13.4 ± 0.1
Demethoxycurcumin	32.0 ± 2.6	24.8 ± 2.4	26.4 ± 8.8	14.0 ± 0.1
Bisdemethoxycurcumin	42.0 ± 6.5	23.2 ± 4.2	30.5 ± 9.7	16.7 ± 0.2

Table 21. IC_{20} values of curcuminoids derivatives on cytotoxicity of leukemic cell lines. The data represent the mean values (μM) ± SD of three independent experiments performed in triplicate.

Turmeric curcuminoids	IC_{20} on leukemic cell lines (μM)			
	K562	U937	HL-60	Molt4
Curcuminoid mixture	9.3 ± 2.2	8.9 ± 1.2	10.1 ± 1.4	8.9 ± 0.3
Pure curcumin	12.2 ± 2.3	8.5 ± 0.3	13.1 ± 6.2	9.2 ± 0.2
Demethoxycurcumin	10.9 ± 1.0	10.0 ± 2.0	13.6 ± 4.0	8.5 ± 0.3
Bisdemethoxycurcumin	14.7 ± 1.0	13.2 ± 1.3	14.8 ± 1.4	10.9 ± 0.2

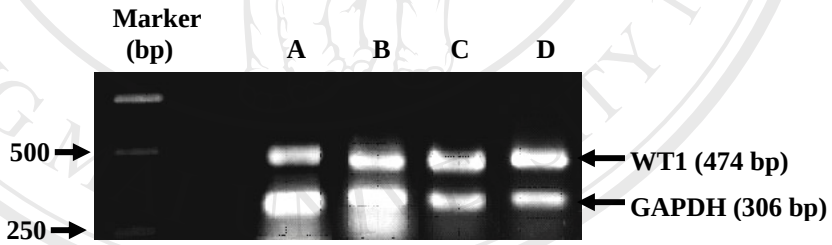

Figure 12. The WT1 and GAPDH mRNA levels in K562, U937, HL-60 and Molt4 cell lines. The WT1 and GAPDH mRNA levels in untreated K562 (A), U937 (B), HL-60 (C), and Molt4 (D) cell lines were determined by RT-PCR. Fifteen microlitre of the PCR products (474 bp for WT1 and 306 bp for GAPDH) were run on 1% agarose gel.

3.3 Effect of turmeric curcuminoids on K562 cell line

3.3.1 Effect of turmeric curcuminoids on K562 cell morphology and viability

To study the effect of 10 µM turmeric curcuminoids on cell morphology and cell viability, K562 cells were observed after 2 days of treatment with commercial grade curcuminoid mixture (Sigma-Aldrich; USA), curcuminoid mixture (in-house curcuminiods), pure curcumin, demethoxycurcumin, and bisdemethoxycurcumin using an inverse compound microscope. There were no differences in cell morphology after 10 µM curcuminoid treatment compared to the vehicle control (0.05% DMSO) (Figure 13). The cytotoxic effect of commercial grade curcuminoid mixture (Sigma-Aldrich; USA), curcuminoid mixture (in-house curcuminiods), pure curcumin, demethoxycurcumin, and bisdemethoxycurcumin on K562 cells for 2 days was then assessed by the trypan blue exclusion method. It was found that 10 µM of five curcuminoids derivatives were not cytotoxic to K562 cells. The cell viabilities were 84, 87, 90, 88 and 85%, respectively. The vehicle control had 90% cell viability.

3.3.2 Effect of curcuminoids derivatives on *WT1* gene expression in K562 cell line

To investigate the effect of turmeric curcuminoids on *WT1* gene expression in K562 cell line, the leukemic cell line was treated with 0.05% DMSO (vehicle control) and with 10 µM of each form of curcuminoids, including commercial grade curcuminoid mixture (Sigma-Aldrich), curcuminoid mixture, pure curcumin, demethoxycurcumin, and bisdemethoxycurcumin. After curcuminoid treatment for 2 days, total RNA extraction (1 µg/reaction) and RT-PCR were carried out. The PCR products (15 µL) were electrophoresed and visualized using scan densitometry, as described in sections 2.6 and 2.7. The experiment was done three times and the WT1 mRNA levels were normalized by the GAPDH mRNA levels. All of the curcuminoids decreased the WT1 mRNA levels in K562 cell lines when compared with the vehicle control by 54, 37, 59, 29, and 39% in response to treatment with commercial grade curcuminoid mixture (Sigma-Aldrich), curcuminoid mixture, pure curcumin, demethoxycurcumin, and bisdemethoxycurcumin, respectively (Figure 14).

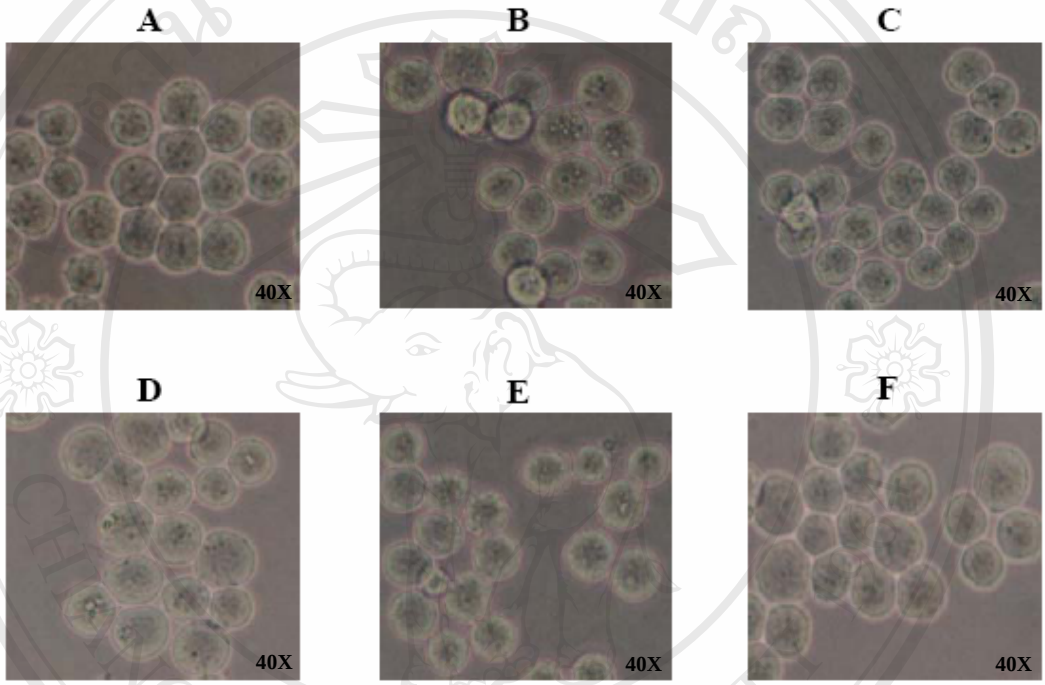

Figure 13. K562 cell morphology after curcuminoid treatments. K562 cells were observed after curcuminoids treatment for 2 days with (A) 0.05% DMSO, (B) commercial grade curcuminoid mixture, (C) in-house curcuminoid mixture, (D) pure curcumin, (E) demethoxycurcumin, and (F) bisdemethoxycurcumin.

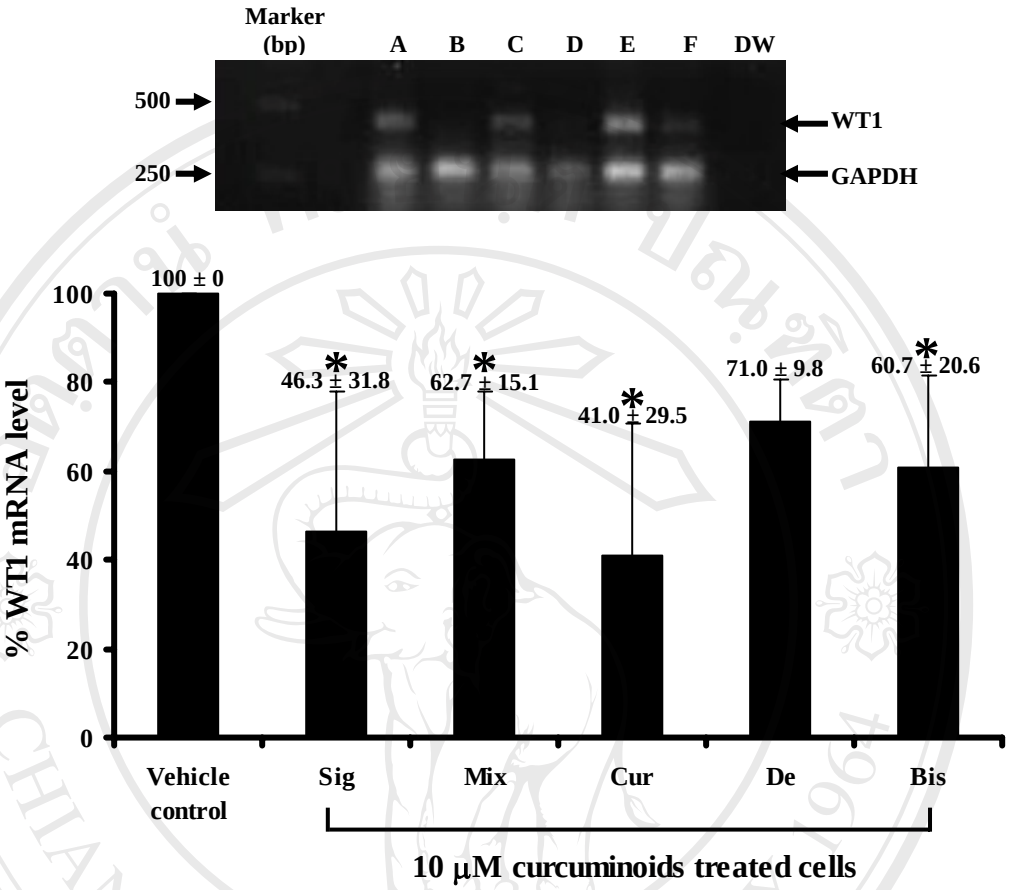

Figure 14. Analysis of the effect of curcuminoid extracts on WT1 mRNA level in K562 cell line. The WT1 and GAPDH mRNA levels following treatment with (A) 0.05% DMSO and 10 μM of (B) commercial grade curcuminoid mixture; Sig, (C) curcuminoid mixture; Mix, (D) pure curcumin; Cur, (E) demethoxycurcumin; De, and (F) bisdemethoxycurcumin; Bis were determined in K562 cells after 2 days by RT-PCR. Fifteen microlitre of the PCR products (474 bp for WT1 and 306 bp for GAPDH) were run on 1% agarose gel. The bands were quantified using a scan densitometer. WT1 mRNA levels were measured and normalized with GAPDH mRNA level. Distilled water (DW) was used as a negative control. Data are the mean values ± standard deviation (SD) of three independent experiments. Asterisks (*) denote values that were significantly different from the vehicle control ($p < 0.05$).

3.3.3 Effect of concentration of pure curcumin on *WT1* gene expression in K562 cell line

According to the results in section 3.3.2, pure curcumin had the strongest inhibitory effect on WT1 mRNA level in K562 cells. To study its effect in a dosage dependent manner, non-toxic doses of pure curcumin (5, 10, and 15 µM) were used. The leukemic cell line was cultured with 0.05% DMSO (vehicle control) or 5, 10, and 15 µM pure curcumin for 2 days. Then, the cells were examined for morphology and viability. Total RNA extraction (1 µg/reaction) and RT-PCR were carried out. The PCR products (15 µL) were electrophoresed and visualized using a scan densitometer, as described in sections 2.6 and 2.7. The experiment was done three times, and the WT1 mRNA levels were normalized by the GAPDH mRNA levels. The morphology of K562 cells after treatment with all concentrations of pure curcumin was not different from the vehicle control (Figure 15). The viabilities of K562 cells after treatment with 5, 10, and 15 µM were 96, 96, and 95%, respectively, while the vehicle control was 96%. Moreover, treatment with 5, 10, and 15 µM of pure curcumin decreased the WT1 mRNA level in a dose dependent manner by 15, 28, and 50%, respectively (Figure 16). In addition, 10 and 15 µM cucumin significantly decreased the WT1 mRNA levels when compared to the level in the vehicle control.

3.3.4 Effect of duration of pure curcumin treatments on *WT1* gene expression in K562 cell line

Pure curcumin had the strongest inhibitory effect on *WT1* gene expression in K562 cells. To evaluate its effect in a time dependent manner, the leukemic cell line was treated with 10 µM of pure curcumin for 1, 2, and 3 days. For the vehicle control, the cells were incubated with 0.05% DMSO for 3 days. After incubation, the cells were examined for morphology and viability. Total RNA extraction (1 µg/reaction) and RT-PCR were carried out. The PCR (15 µL) products were electrophoresed and visualized using a scan densitometer, as described in sections 2.6 and 2.7. The experiment was done three times, and the WT1 mRNA levels were normalized by the GAPDH mRNA levels. The morphology of K562 cells after treatment with pure curcumin was not different to the vehicle control cells (Figure 17). The cell viabilities of K562 cells in vehicle control group and after pure curcumin treatment for 1, 2, and 3

days were 94, 98, 96, and 93%, respectively. Moreover, treatment with pure curcumin at all incubation times significantly decreased the WT1 mRNA levels in a time dependent manner by 37, 48, and 54%, respectively (Figure 18).

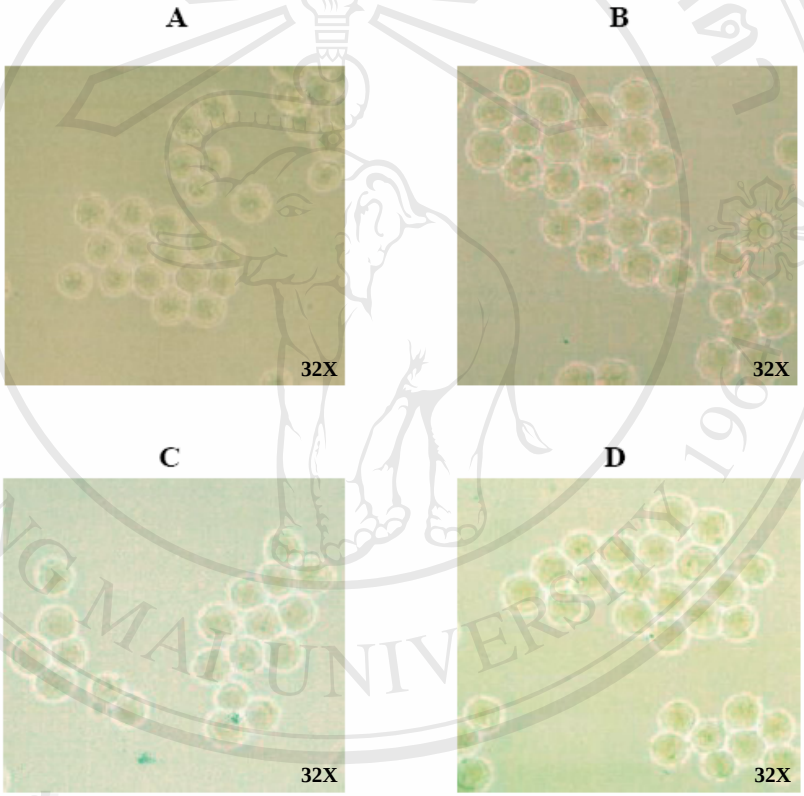

Figure 15. K562 cell morphology after pure curcumin treatment with 5, 10, and 15 µM. Cell morphology of K562 was determined after 48 h of treatment with (A) 0.05% DMSO, (B) 5 µM pure curcumin, (C) 10 µM pure curcumin, and (D) 15 µM pure curcumin.

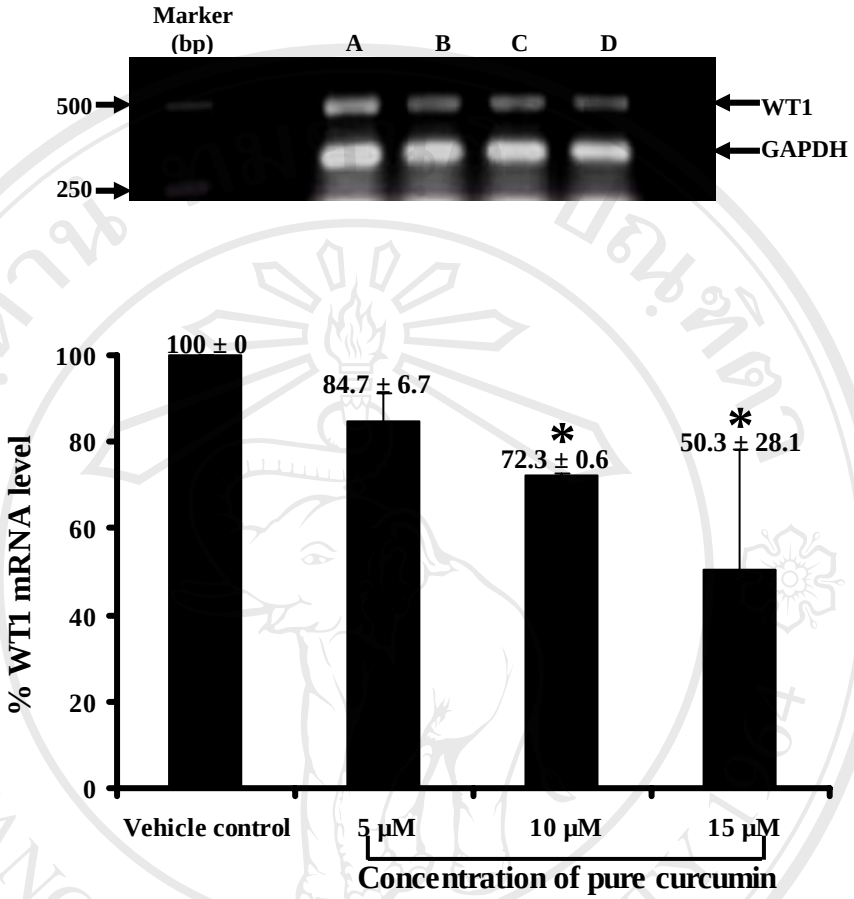

Figure 16. The WT1 mRNA levels in K562 cell line cultured in 5, 10, and 15 µM of pure curcumin for 2 days. The WT1 and GAPDH mRNA levels following treatment with (A) 0.05% DMSO and pure curcumin at a concentration (B) 5 µM, (C) 10 µM, and (D) 15 µM were determined in K562 cells after 2 days by RT-PCR. Fifteen microlitre of the PCR products were electrophoresed on 1% agarose gel. The bands were quantified using a scan densitometer. WT1 mRNA level was measured and normalized with GAPDH mRNA level. Distilled water (DW) was used as a negative control. Data are the mean value ± SD of three independent experiments. Asterisks (*****) denote values that were significantly different from the vehicle control ($p < 0.05$).

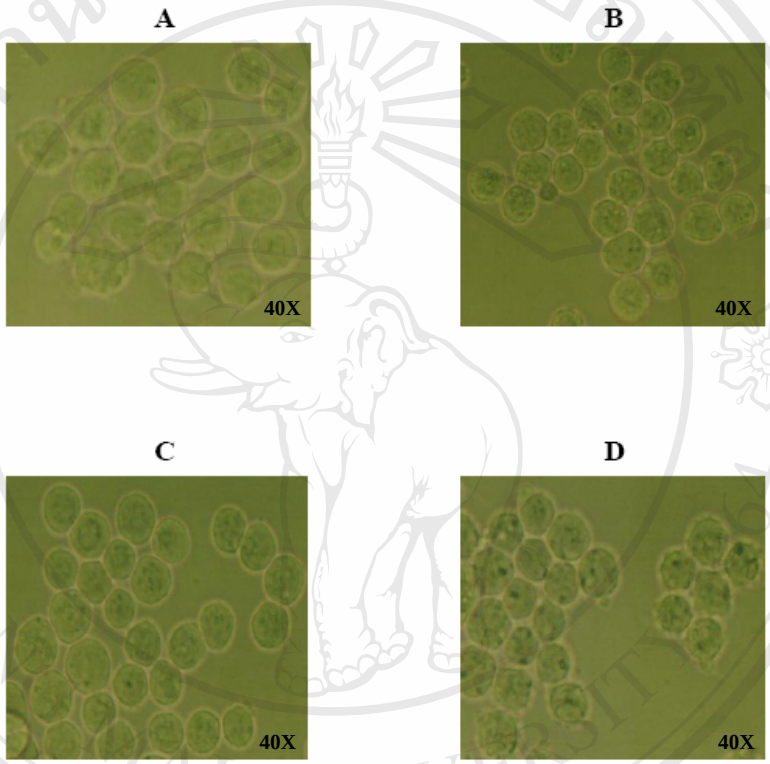

Figure 17. K562 cell morphology after treatment with 10 µM of pure curcumin for 1, 2, and 3 days. Cell morphology of K562 was determined after treatment with (A) 0.05% DMSO for 3 days and 10 µM pure curcumin for (B) 1 day, (C) 2 days, and (D) 3 days.

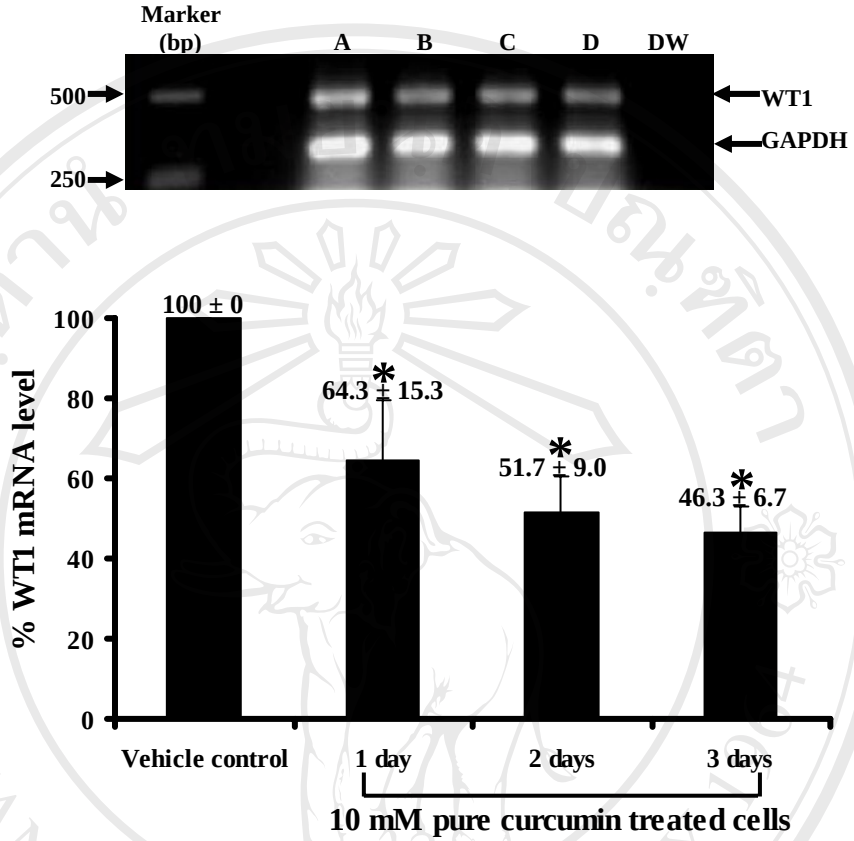

Figure 18. The WT1 mRNA levels in K562 cell line cultured in 10 µM of pure curcumin for 1, 2, and 3 days. The WT1 and GAPDH mRNA levels following treatment with (A) 0.05% DMSO for 3 days and 10 µM pure curcumin for (B) 1 day, (C) 2 days, and (D) 3 days were determined in K562 by RT-PCR. Fifteen microlitre of the PCR products were electrophoresed on 1% agarose gel. The bands were quantified using a scan densitometer. WT1 mRNA level was measured and normalized with GAPDH mRNA level. Distilled water (DW) was used as a negative control. Data are the mean value ± SD of three independent experiments. Asterisks (*****) denote values that were significantly different from the vehicle control ($p < 0.05$).

3.4 Effect of turmeric curcuminoids on U937 cell line

3.4.1 Effect of turmeric curcuminoids on U937 cell morphology and viability

To study the effect of 10 µM turmeric curcuminoids on cell morphology and cell viability, U937 cells were observed after 2 days of treatment with commercial grade curcuminoid mixture (Sigma-Aldrich), curcuminoid mixture, pure curcumin, demethoxycurcumin, and bisdemethoxycurcumin using an inverse compound microscope. After curcuminoids treatment, cell morphology was examined. There were no differences in cell morphology after 10 µM curcuminoid treatment compared to the vehicle control (0.05% DMSO), as shown in figure 19. The cytotoxic effect of commercial grade curcuminoid mixture (Sigma-Aldrich), curcuminoid mixture, pure curcumin, demethoxycurcumin, and bisdemethoxycurcumin on U937 cells for 2 days was then assessed by the trypan blue exclusion method. It was found that 10 µM of five curcuminoids derivatives were not cytotoxic in U937 cell. The cell viabilities were 94, 94, 93, 97, and 97%, respectively. The vehicle control had 97% cell viability.

3.4.2 Effect of curcuminoids derivatives on *WT1* gene expression in U937 cell line

To investigate the effect of turmeric curcuminoids on *WT1* gene expression in U937 cell line, the leukemic cells were treated with 0.05% DMSO (vehicle control) and with 10 µM of each form of curcuminoids, including commercial grade curcuminoid mixture (Sigma-Aldrich), curcuminoid mixture, pure curcumin, demethoxycurcumin, and bisdemethoxycurcumin. After curcuminoid treatment for 2 days, total RNA extraction (1 µg/reaction) and RT-PCR were carried out. The PCR products (15 µL) were electrophoresed and visualized using a scan densitometer, as described in sections 2.6 and 2.7. The experiment was done three times, and the WT1 mRNA levels were normalized by the GAPDH mRNA levels. All of the curcuminoids decreased the WT1 mRNA levels in U937 cell lines when compared with the vehicle control by 25, 8, 43, 18, and 28% in response to treatment with commercial grade curcuminoid mixture (Sigma-Aldrich), curcuminoid mixture, pure curcumin, demethoxycurcumin, and bisdemethoxycurcumin, respectively (Figure 20).

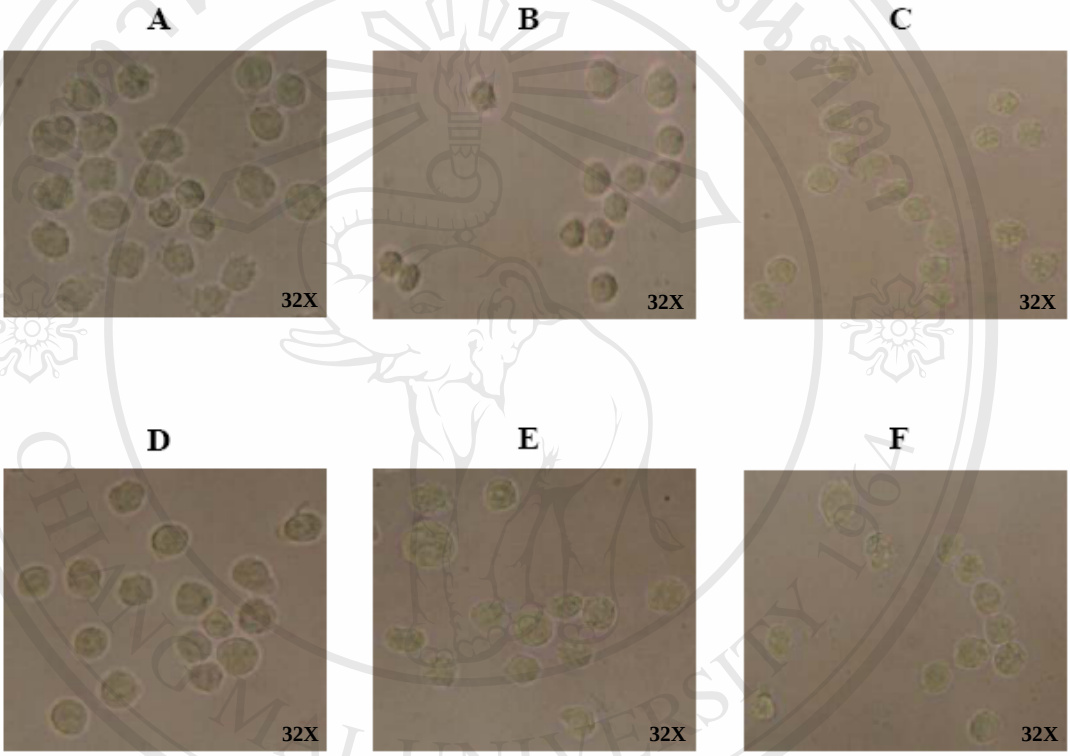

Figure 19. U937 cell morphology of after curcuminoid treatments. U937 cells were observed after curcuminoids treatment for 2 days with (A) 0.05% DMSO, (B) commercial grade curcuminoid mixture, (C) in-house curcuminoid mixture, (D) pure curcumin, (E) demethoxycurcumin, and (F) bisdemethoxycurcumin.

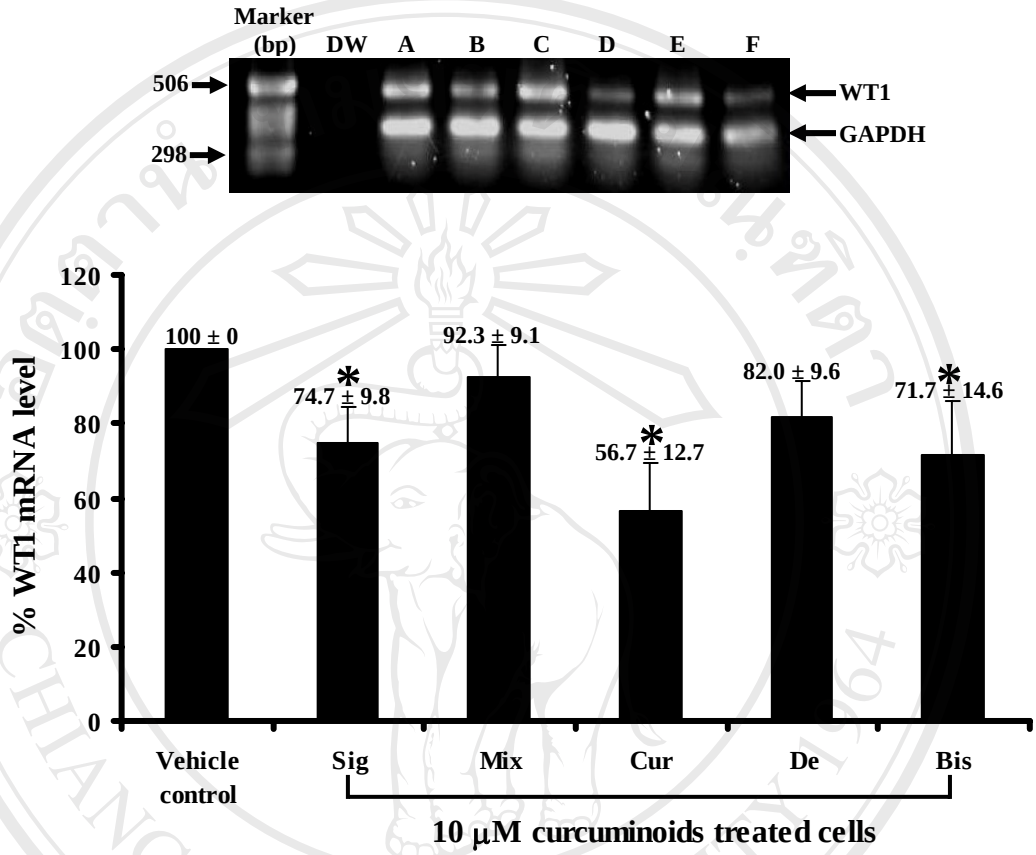

Figure 20. Analysis of the effect of curcuminoid extracts on WT1 mRNA level in U937 cell line. The WT1 and GAPDH mRNA levels following treatment with (A) 0.05% DMSO and 10 μM of (B) commercial grade curcuminoid mixture; Sig, (C) curcuminoid mixture; Mix, (D) pure curcumin; Cur, (E) demethoxycurcumin; De, and (F) bisdemethoxycurcumin; Bis were determined in U937 cells after 2 days by RT-PCR. Fifteen microlitre of the PCR products (474 bp for WT1 and 306 bp for GAPDH) were run on 1% agarose gel. The bands were quantified using a scan densitometer. WT1 mRNA levels were measured and normalized with GAPDH mRNA level. Distilled water (DW) was used as a negative control. Data are the mean value ± standard deviation (SD) of three independent experiments. Asterisks (*****) denote values that were significantly different from the vehicle control ($p < 0.05$).

3.4.3 Effect of concentrations of pure curcumin on *WT1* gene expression in U937 cell line

According to the results in section 3.4.2, pure curcumin had the strongest inhibitory effect on WT1 mRNA level in U937 cells. To study its effect in a dose dependent manner, non-toxic doses of pure curcumin (5, 10 and, 15 µM) were used. The leukemic cell line was cultured with 0.05% DMSO (vehicle control) or 5, 10, and 15 µM pure curcumin for 2 days. Then, the cells were examined for morphology and viability. Total RNA extraction (1 µg/reaction) and RT-PCR were carried out. The PCR products (15 µL) were electrophoresed and visualized using a scan densitometer, as described in sections 2.6 and 2.7. The experiment was done three times, and the WT1 mRNA levels were normalized by the GAPDH mRNA level. The morphology of U937 cells after treatment with all concentrations of pure curcumin was not different from the vehicle control (Figure 21). The viability of U937 cells after treatment with 5, 10, and 15 µM were 93, 91, and 92%, respectively, while the vehicle control was 94%. Moreover, treatment with 5, 10, and 15 µM of pure curcumin decreased the WT1 mRNA level in a dose dependent manner by 10, 17, and 19%, respectively (Figure 22). In addition, all concentrations of cucumin significantly decreased the WT1 mRNA levels when compare to the level in the vehicle control.

3.4.4 Effect of duration of pure curcumin treatments on *WT1* gene expression in U937 cell line

According to the results in section 3.4.2, pure curcumin had the strongest inhibitory effect on *WT1* gene expression in U937 cells. To evaluate its effect in a time dependent manner, the leukemic cell lines were treated with 10 µM of pure curcumin for 1, 2 and, 3 days. For the vehicle control, the cells were incubated with 0.05% DMSO for 3 days. After incubation, the cells were examined for morphology and viability. Total RNA extraction (1 µg/reaction) and RT-PCR were carried out. The PCR products (15 µL) were electrophoresed and visualized using a scan densitometer, as described in sections 2.6 and 2.7. The experiment was done three times, and the WT1 mRNA levels were normalized by the GAPDH mRNA level. The morphology of U937 cells after treatment with pure curcumin was not different to that of the vehicle control cells (Figure 23). The cell viability of U937 cells in the vehicle control group

and after pure curcumin treatment for 1, 2, and 3 days were 95, 88, 89, and 87%, respectively. In addition, treatment with pure curcumin for all incubation times significantly decreased the WT1 mRNA levels in a time dependent manner by 13, 33, and 64%, respectively (Figure 24). Moreover, significantly different levels of WT1 mRNA were found after 2 and 3 days of pure curcumin treatment.

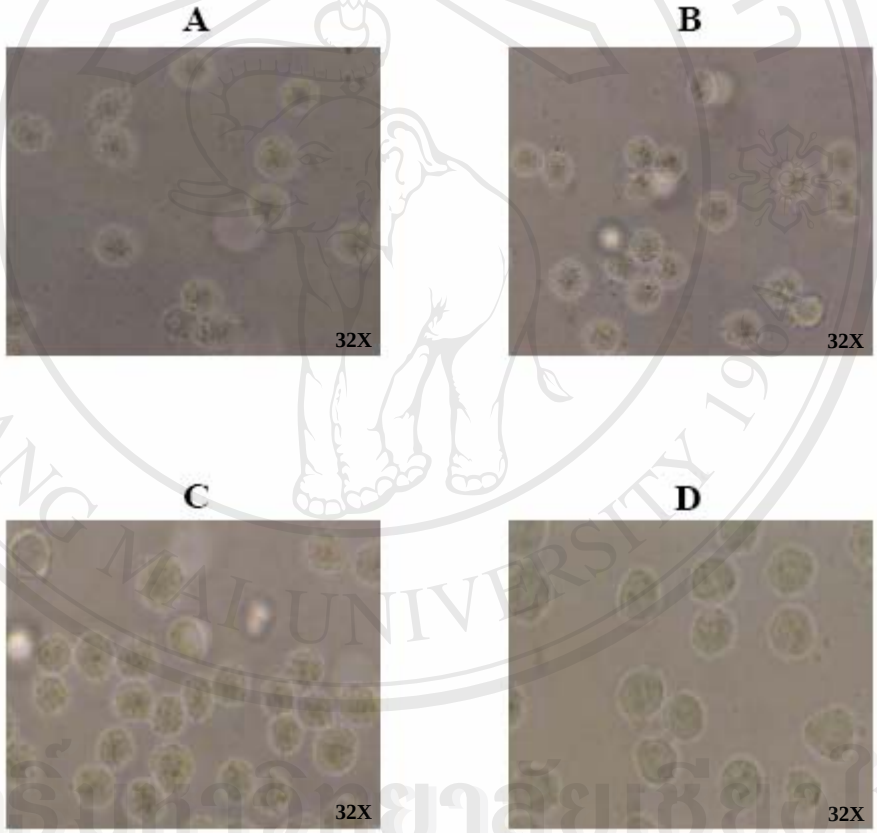

Figure 21. U937 cell morphology after pure curcumin treatment with 5, 10, and 15 µM. Cell morphology of U937 was determined after 48 h of treatment with (A) 0.05% DMSO, (B) 5 µM pure curcumin, (C) 10 µM pure curcumin, and (D) 15 µM pure curcumin.

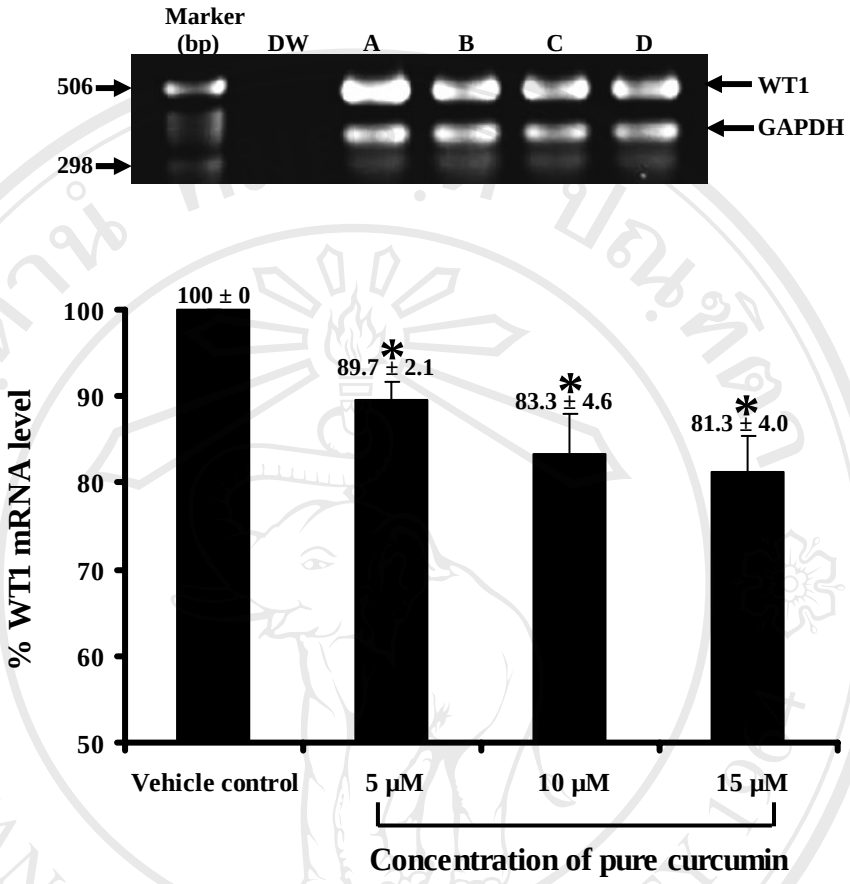

Figure 22. The WT1 mRNA levels in U937 cell line cultured in 5, 10, and 15 µM of pure curcumin for 2 days. The WT1 and GAPDH mRNA levels following treatment with (A) 0.05% DMSO and pure curcumin at a concentration (B) 5 µM, (C) 10 µM, and (D) 15 µM were determined in U937 cells after 2 days by RT-PCR. Fifteen microlitre of the PCR products were electrophoresed on 1% agarose gel. The bands were quantified using a scan densitometer. WT1 mRNA level was measured and normalized with GAPDH mRNA level. Distilled water (DW) was used as a negative control. Data are the mean value ± SD of three independent experiments. Asterisks (*****) denote values that were significantly different from the vehicle control ($p < 0.05$).

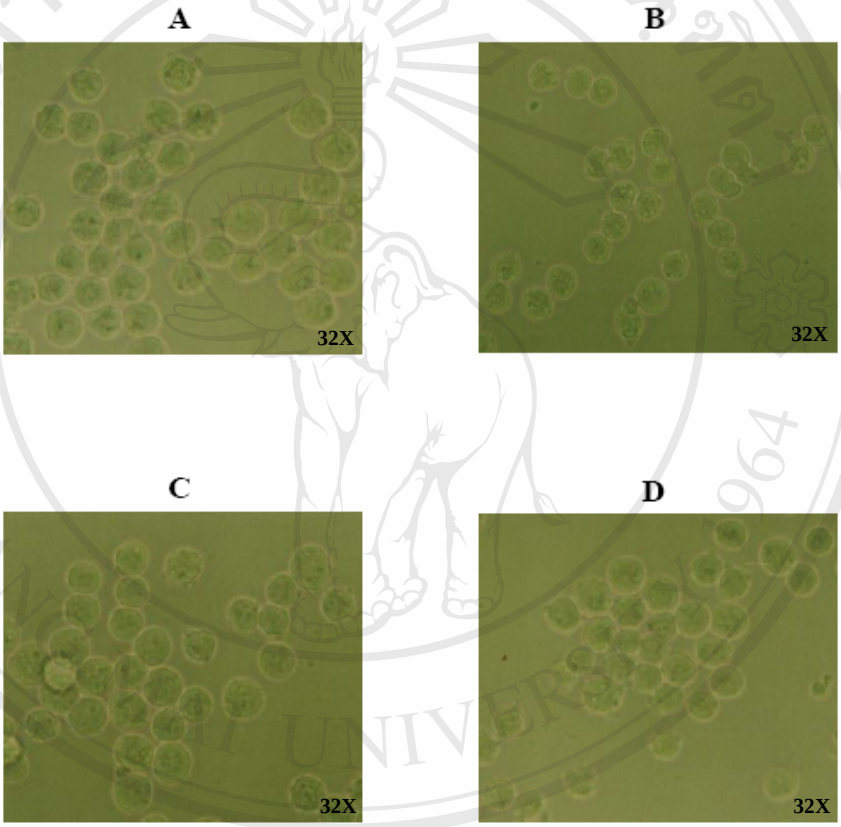

Figure 23. U937 cell morphology after treatment with 10 µM of pure curcumin for 1, 2, and 3 days. Cell morphology of U937 was determined after treatment with (A) 0.05% DMSO for 3 days and 10 µM pure curcumin for (B) 1 day, (C) 2 days, and (D) 3 days.

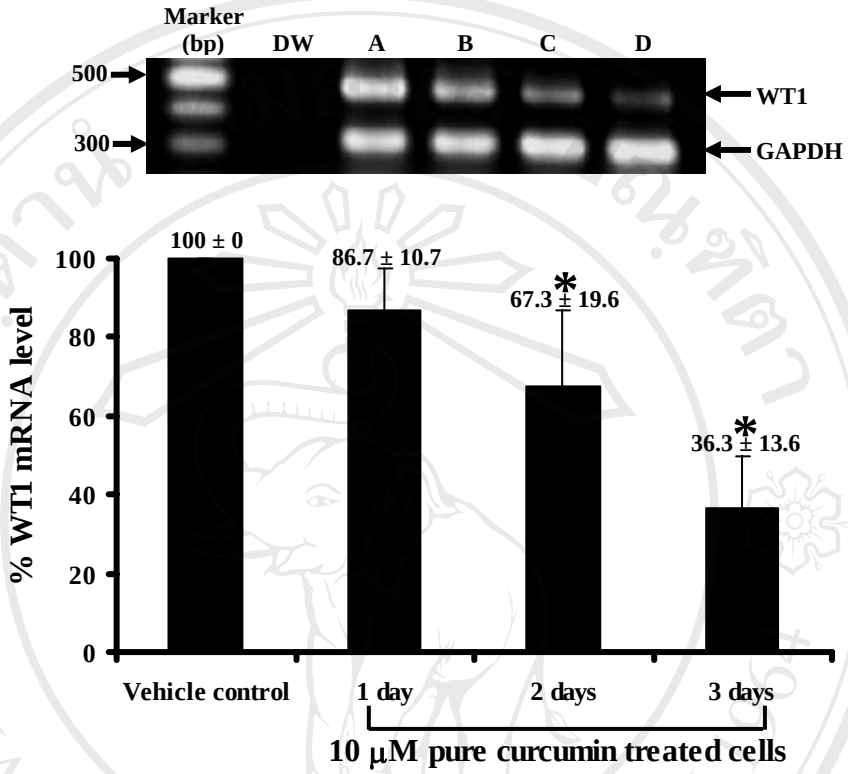

Figure 24. The WT1 mRNA levels in U937 cell line cultured in 10 μM of pure curcumin for 1, 2, and 3 days. The WT1 and GAPDH mRNA levels following treatment with (A) 0.05% DMSO for 3 days and 10 μM pure curcumin for (B) 1 day, (C) 2 days, and (D) 3 days were determined in U937 by RT-PCR. Fifteen microlitre of the PCR products were electrophoresed on 1% agarose gel. The bands were quantified using a scan densitometer. WT1 mRNA level was measured and normalized with GAPDH mRNA level. Distilled water (DW) was used as a negative control. Data are the mean value ± SD of three independent experiments. Asterisks (*****) denote values that were significantly different from the vehicle control ($p < 0.05$).

3.5 Effect of turmeric curcuminoids on HL-60 cell line

3.5.1 Effect of turmeric curcuminoids on HL-60 cell morphology and viability

To study the effect of 10 µM turmeric curcuminoids on cell morphology and cell viability, HL-60 cells were observed after 2 days of treatment with commercial grade curcuminoid mixture (Sigma-Aldrich), curcuminoid mixture, pure curcumin, demethoxycurcumin, and bisdemethoxycurcumin using an inverse compound microscope. After curcuminoids treatment, cell morphology was examined. There were no differences in cell morphology after 10 µM curcuminoid treatment compared to the vehicle control (0.05% DMSO) as shown in figure 25. The cytotoxic effect of commercial grade curcuminoid mixture (Sigma-Aldrich), curcuminoid mixture, pure curcumin, demethoxycurcumin, and bisdemethoxycurcumin on HL-60 cells for 2 days was then assessed by the trypan blue exclusion method. It was found that 10 µM of five curcuminoids derivatives were not cytotoxic to HL-60 cells. The cell viabilities were 88, 86, 95, 86, and 86%, respectively. The vehicle control had 93% cell viability.

3.5.2 Effect of curcuminoids derivatives on *WT1* gene expression in HL-60 cell line

To investigate the effect of turmeric curcuminoids on *WT1* gene expression in HL-60 cell line, the leukemic cell line was treated with 0.05% DMSO (vehicle control) and with 10 µM of each form of curcuminoids, including commercial grade curcuminoid mixture (Sigma-Aldrich), curcuminoid mixture, pure curcumin, demethoxycurcumin, and bisdemethoxycurcumin. After curcuminoid treatment for 2 days, total RNA extraction (1 µg/reaction) and RT-PCR were carried out. The PCR products (15 µL) were electrophoresed and visualized using a scan densitometer, as described in sections 2.6 and 2.7. The experiment was done three times, and the WT1 mRNA levels were normalized by the GAPDH mRNA levels. The result showed that all of the curcuminoids decreased the WT1 mRNA levels in HL-60 cell lines when compared with vehicle control by 16, 7, 37, 14, and 15% in response to treatment with commercial grade curcuminoid mixture (Sigma-Aldrich), curcuminoid mixture, pure curcumin, demethoxycurcumin, and bisdemethoxycurcumin, respectively (Figure 26).

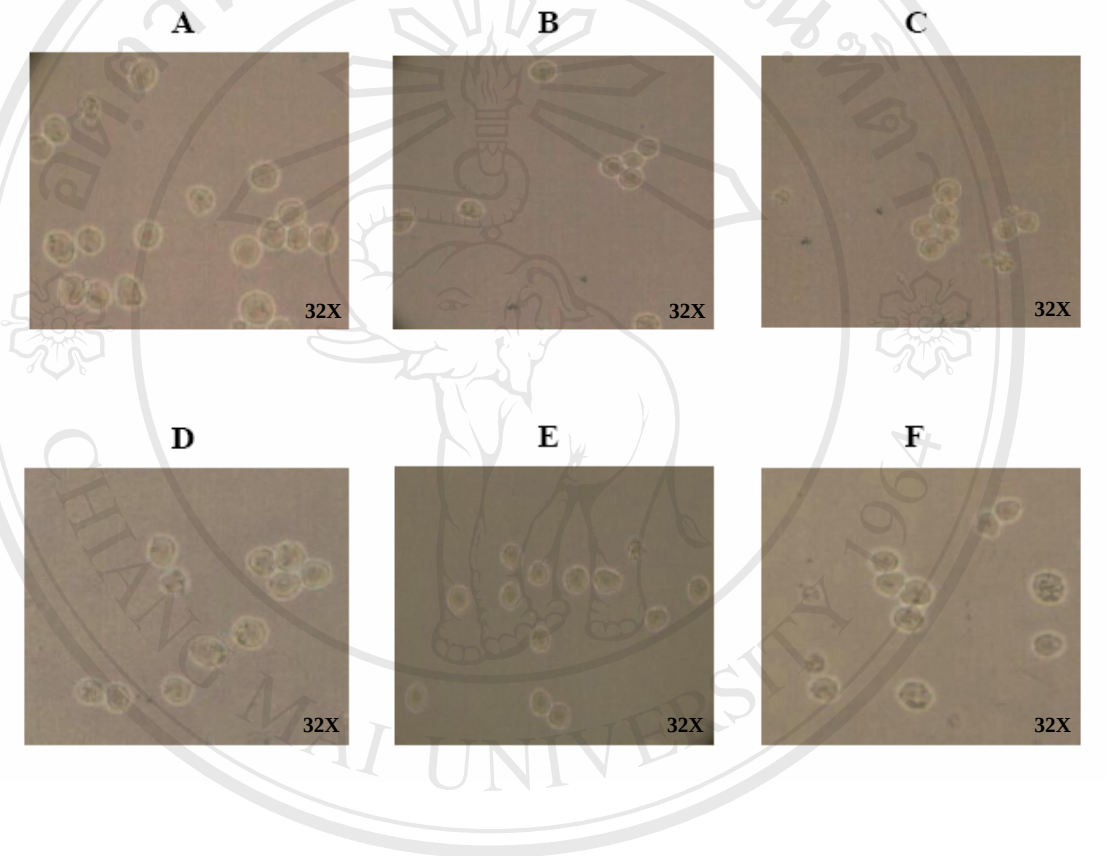

Figure 25. HL-60 cell morphology after curcuminoid treatments. HL-60 cells were observed after curcuminoids treatment for 2 days with (A) 0.05% DMSO, (B) commercial grade curcuminoid mixture, (C) in-house curcuminoid mixture, (D) pure curcumin, (E) demethoxycurcumin, and (F) bisdemethoxycurcumin.

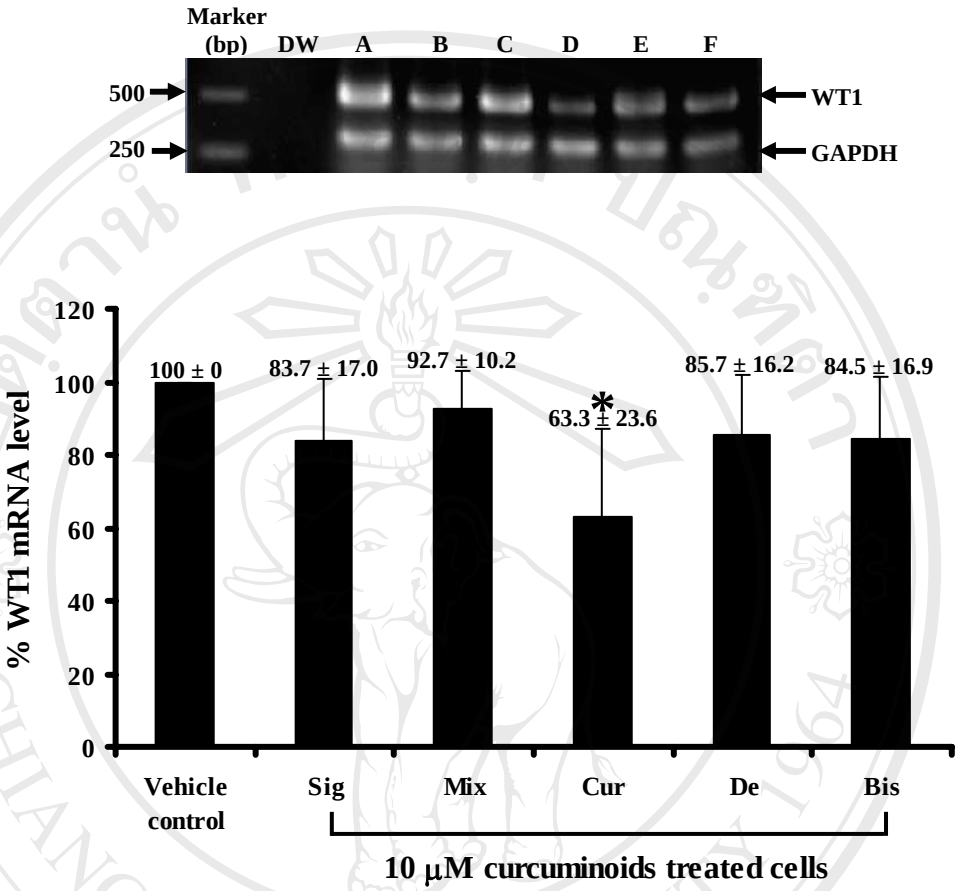

Figure 26. Analysis of the effect of curcuminoid extracts on WT1 mRNA level in HL-60 cell line. The WT1 and GAPDH mRNA levels following treatment with (A) 0.05% DMSO and 10 μM of (B) commercial grade curcuminoid mixture; Sig, (C) curcuminoid mixture; Mix, (D) pure curcumin; Cur, (E) demethoxycurcumin; De, and (F) bisdemethoxycurcumin; Bis were determined in HL-60 cells after 2 days by RT-PCR. Fifteen microlitre of the PCR products (474 bp for WT1 and 306 bp for GAPDH) were run on 1% agarose gel. The bands were quantified using a scan densitometer. WT1 mRNA levels were measured and normalized with GAPDH mRNA level. Distilled water (DW) was used as a negative control. Data are the mean value ± standard deviation (SD) of three independent experiments. Asterisks (*****) denote values that were significantly different from the vehicle control ($p < 0.05$).

3.5.3 Effect of concentrations of pure curcumin on *WT1* gene expression in HL-60 cell line

According to the results in section 3.5.2, pure curcumin had the strongest inhibitory effect on WT1 mRNA level in HL-60 cells. To study its effect in a dose dependent manner, non-toxic doses of pure curcumin (5, 10, and 15 µM) were used. The leukemic cell lines were cultured with 0.05% DMSO (vehicle control) or 5, 10, and 15 µM pure curcumin for 2 days. Then, the cells were examined for morphology and viability. Total RNA extraction (1 µg/reaction) and RT-PCR were carried out. The PCR products (15 µL) were electrophoresed and visualized using a scan densitometer, as described in sections 2.6 and 2.7. The experiment was done three times, and the WT1 mRNA levels were normalized by the GAPDH mRNA level. The morphology of HL-60 cells after treatment with all concentrations of pure curcumin was not different from the vehicle control (Figure 27). The viability of HL-60 cells after treatment with 5, 10 and 15 µM were 95, 95, and 84%, respectively, while the vehicle control was 96%. Moreover, treatment with 5, 10, and 15 µM of pure curcumin decreased the WT1 mRNA level in a dose dependent manner by 5, 11, and 18%, respectively (Figure 28).

3.5.4 Effect of duration of pure curcumin treatments on *WT1* gene expression in HL-60 cell line

According to the results in section 3.5.2, pure curcumin had the strongest inhibitory effect on *WT1* gene expression in HL-60 cells. To evaluate its effect in a time dependent manner, the leukemic cell lines were treated with 10 µM of pure curcumin for 1, 2, and 3 days. For the vehicle control, the cells were incubated with 0.05% DMSO for 3 days. After incubation, the cells were examined for morphology and viability. Total RNA extraction (1 µg/reaction) and RT-PCR were carried out. The PCR products (15 µL) were electrophoresed and visualized using a scan densitometer, as described in sections 2.6 and 2.7. The experiment was done three times, and the WT1 mRNA levels were normalized by the GAPDH mRNA level. The morphology of HL-60 cells after treatment with pure curcumin was not different to the vehicle control cells (Figure 29). The cell viability of HL-60 cells in vehicle control group and after pure curcumin treatment for 1, 2, and 3 days were 97, 95, 96, and 94%, respectively. In

addition, treatment with pure curcumin for all incubation times significantly decreased the WT1 mRNA levels in a time dependent manner by 4, 8, and 30%, respectively (Figure 30). Moreover, a significantly different level of WT1 mRNA was found after 2 and 3 days of pure curcumin treatment.

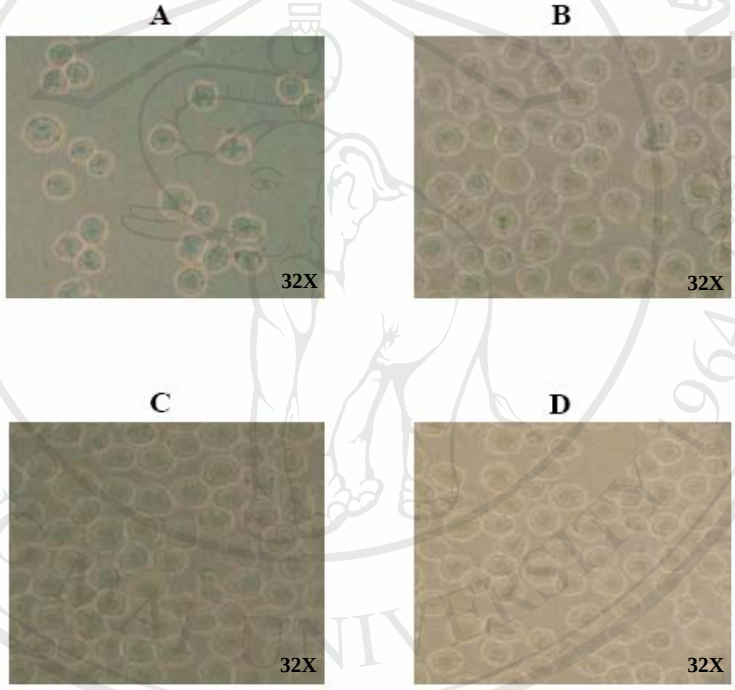

Figure 27. HL-60 cell morphology after pure curcumin treatment with 5, 10, and 15 µM. Cell morphology of HL-60 was examined after 48 h of treatment with (A) 0.05% DMSO, (B) 5 µM pure curcumin, (C) 10 µM pure curcumin, and (D) 15 µM pure curcumin.

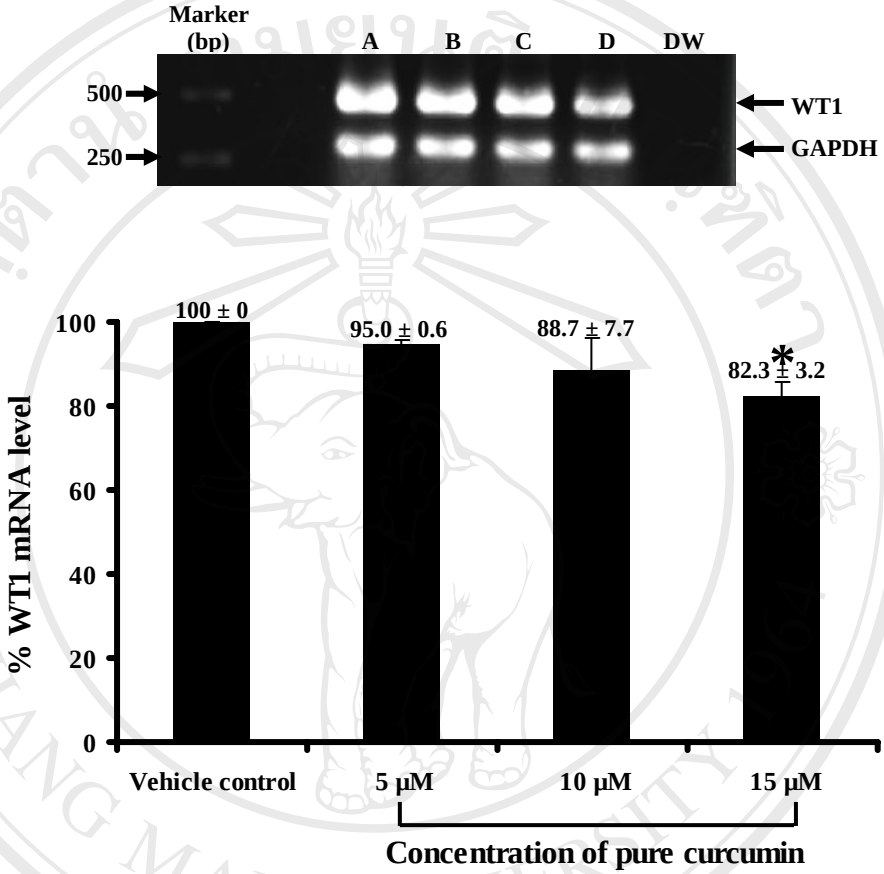

Figure 28. The WT1 mRNA levels in HL-60 cell line cultured in 5, 10, and 15 µM of pure curcumin for 2 days. The WT1 and GAPDH mRNA levels following treatment with (A) 0.05% DMSO and pure curcumin at a concentration (B) 5 µM, (C) 10 µM, and (D) 15 µM were determined in HL-60 cells after 2 days by RT-PCR. Fifteen microlitre of the PCR products were electrophoresed on 1% agarose gel. The bands were quantified using a scan densitometer. WT1 mRNA level was measured and normalized with GAPDH mRNA level. Distilled water (DW) was used as a negative control. Data are the mean value ± SD of three independent experiments. Asterisks (*****) denote values that were significantly different from the vehicle control ($p < 0.05$).

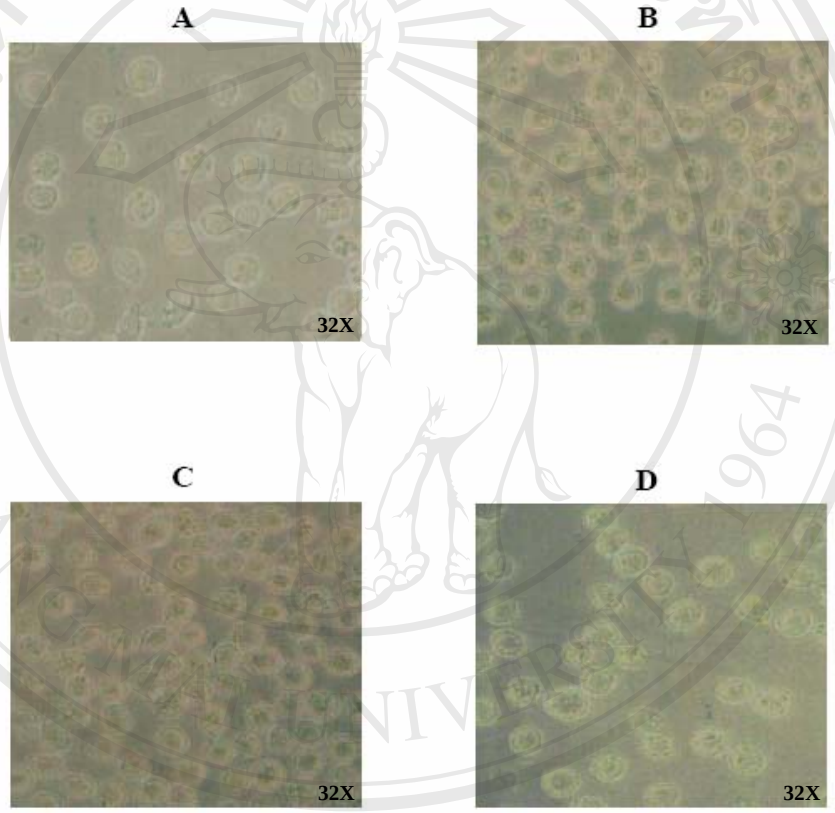

Figure 29. HL-60 cell morphology of after treatment with 10 µM of pure curcumin for 1, 2, and 3 days. Cell morphology of HL-60 was examined after treatment with (A) 0.05% DMSO for 3 days and 10 µM pure curcumin for (B) 1 day, (C) 2 days, and (D) 3 days.

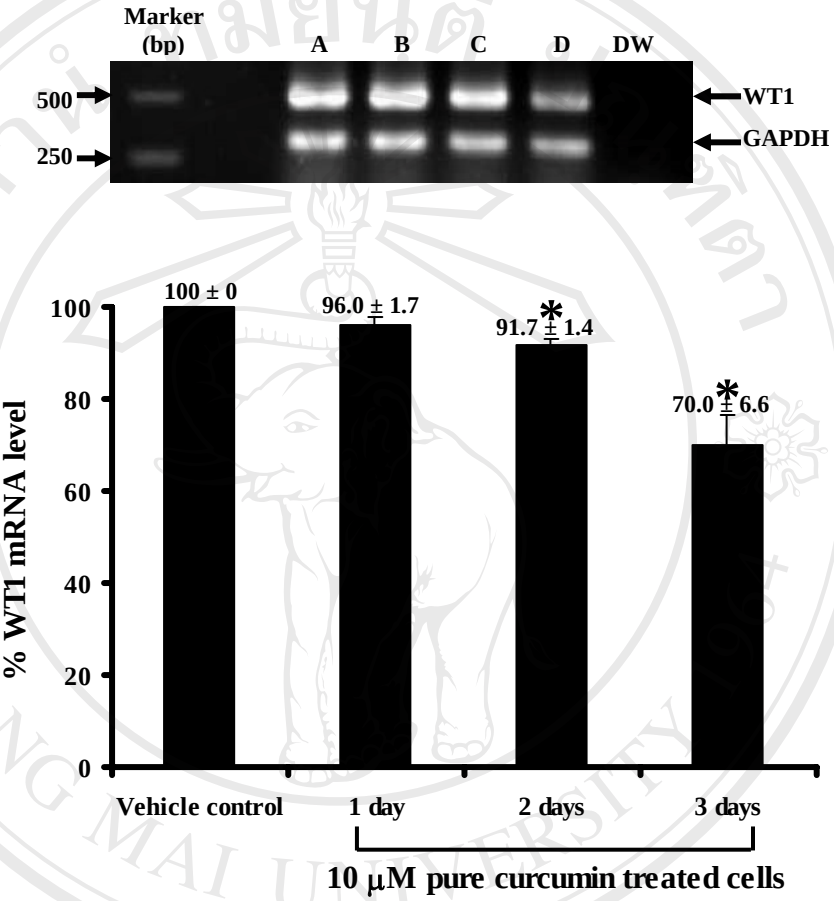

Figure 30. The WT1 mRNA levels in HL-60 cell line cultured in 10 μM of pure curcumin for 1, 2, and 3 days. The WT1 and GAPDH mRNA levels following treatment with (A) 0.05% DMSO for 3 days and 10 μM pure curcumin for (B) 1 day, (C) 2 days, and (D) 3 days were determined in HL-60 by RT-PCR. Fifteen microlitre of the PCR products were electrophoresed on 1% agarose gel. The bands were quantified using a scan densitometer. WT1 mRNA level was measured and normalized with GAPDH mRNA level. Distilled water (DW) was used as a negative control. Data are the mean value ± SD of three independent experiments. Asterisks (*****) denote values that were significantly different from the vehicle control ($p < 0.05$).

3.6 Effect of turmeric curcuminoids on Molt4 cell line

3.6.1 Effect of turmeric curcuminoids on Molt4 cell morphology and viability

To study the effect of 10 µM turmeric curcuminoids on cell morphology and cell viability, Molt4 cells were observed after 2 days of treatment with commercial grade curcuminoid mixture (Sigma-Aldrich), curcuminoid mixture, pure curcumin, demethoxycurcumin, and bisdemethoxycurcumin using an inverse compound microscope. After curcuminoids treatment, cell morphology was examined. There were no differences in cell morphology after 10 µM curcuminoids treatment compared to the vehicle control (0.05% DMSO) as shown in figure 31. The cytotoxic effect of commercial grade curcuminoid mixture (Sigma-Aldrich), curcuminoid mixture, pure curcumin, demethoxycurcumin, and bisdemethoxycurcumin on Molt4 cells after 2 days was then assessed by the trypan blue exclusion method. It was found that 10 µM of five curcuminoids derivatives were not cytotoxic to Molt4 cells. The cell viabilities were 94, 94, 95, 94, and 93%, respectively. The vehicle control had 98% cell viability.

3.6.2 Effect of curcuminoids derivatives on *WT1* gene expression in Molt4 cell line

To investigate the effect of turmeric curcuminoids on *WT1* gene expression in Molt4 cell line, the leukemic cell line was treated with 0.05% DMSO (vehicle control) and with 10 µM of each form of curcuminoids, including commercial grade curcuminoid mixture (Sigma-Aldrich), curcuminoid mixture, pure curcumin, demethoxycurcumin, and bisdemethoxycurcumin. After curcuminoid treatment for 2 days, total RNA extraction (1 µg/reaction) and RT-PCR were carried out. The PCR products (15 µL) were electrophoresed and visualized using a scan densitometer, as described in sections 2.6 and 2.7. The experiment was done three times, and the WT1 mRNA levels were normalized by the GAPDH mRNA levels. All of the curcuminoids decreased the WT1 mRNA levels in Molt4 cell lines when compared with vehicle control by 2.2, 0.8, 17.2, 3.0, and 3.2% in response to treatment with commercial grade curcuminoid mixture (Sigma-Aldrich), curcuminoid mixture, pure curcumin, demethoxycurcumin, and bisdemethoxycurcumin, respectively (Figure 32).

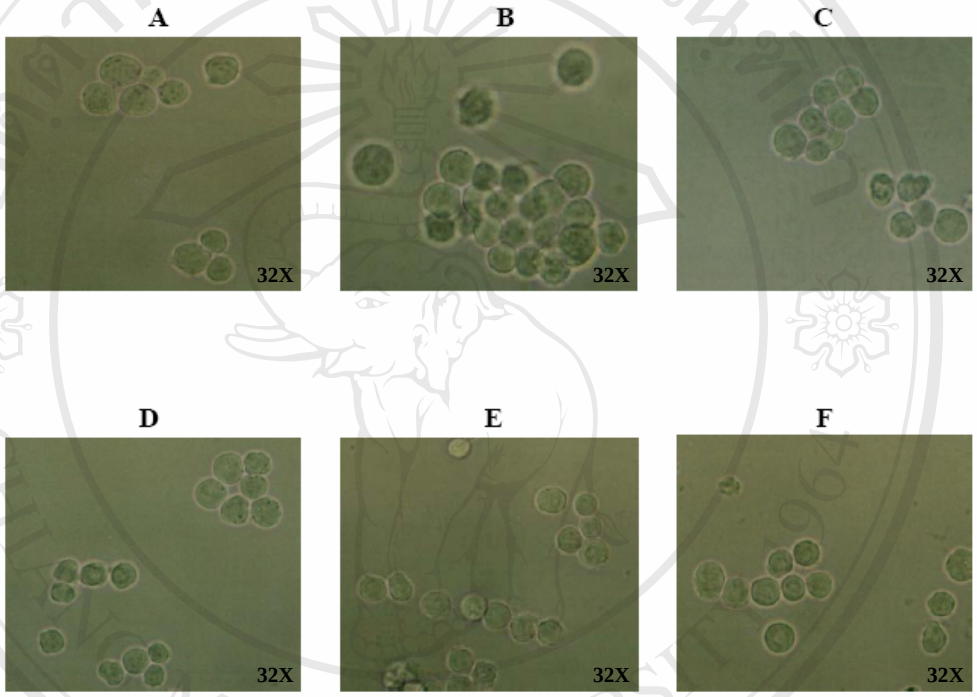

Figure 31. Molt4 cell morphology after curcuminoid treatments. Molt4 cells were examined after curcuminoids treatment for 2 days with (A) 0.05% DMSO, (B) commercial grade curcuminoid mixture, (C) in-house curcuminoid mixture, (D) pure curcumin, (E) demethoxycurcumin, and (F) bisdemethoxycurcumin.

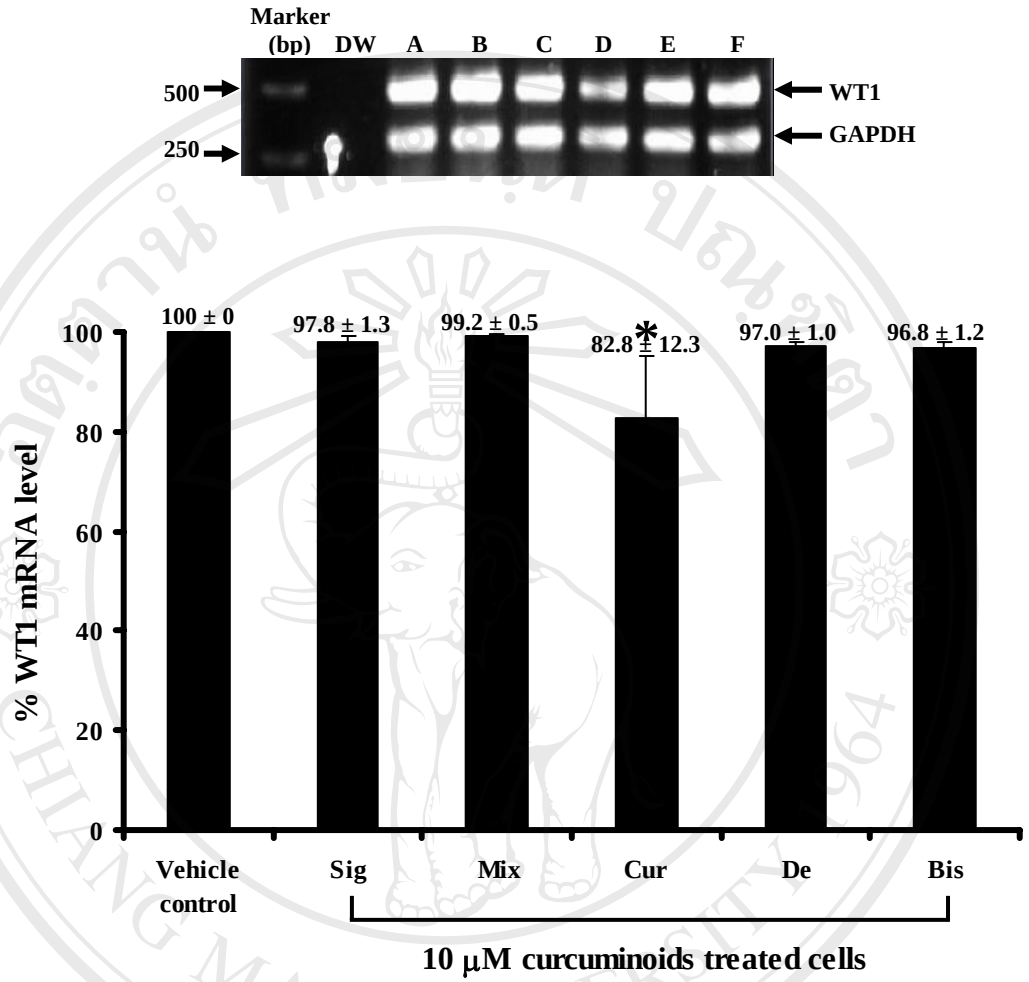

Figure 32. Analysis of the effect of curcuminoid extracts on WT1 mRNA level in Molt4 cell line. The WT1 and GAPDH mRNA levels following treatment with (A) 0.05% DMSO and 10 μM of (B) commercial grade curcuminoid mixture; Sig, (C) curcuminoid mixture; Mix, (D) pure curcumin; Cur, (E) demethoxycurcumin; De, and (F) bisdemethoxycurcumin; Bis were determined in Molt4 cells after 2 days by RT-PCR. Fifteen microlitre of the PCR products (474 bp for WT1 and 306 bp for GAPDH) were run on 1% agarose gel. The bands were quantified using a scan densitometer. WT1 mRNA levels were measured and normalized with GAPDH mRNA level. Distilled water (DW) was used as a negative control. Data are the mean value ± standard deviation (SD) of three independent experiments. Asterisks (*****) denote values that were significantly different from the vehicle control ($p < 0.05$).

3.6.3 Effect of concentrations of pure curcumin on *WT1* gene expression in Molt4 cell line

According to the results in section 3.6.2, pure curcumin had the strongest inhibitory effect on WT1 mRNA level in Molt4 cell. To study its effect in a dose dependent manner, non-toxic doses of pure curcumin (5, 10, and 15 µM) were used. The leukemic cell line was cultured with 0.05% DMSO (vehicle control) or 5, 10, and 15 µM pure curcumin for 2 days. Then, the cells were examined for morphology and viability. Total RNA extraction (1 µg/reaction) and RT-PCR were carried out. The PCR products (15 µL) were electrophoresed and visualized using a scan densitometer, as described in sections 2.6 and 2.7. The experiment was done three times, and the WT1 mRNA levels were normalized by the GAPDH mRNA level. The morphology of Molt4 cells after treatment with all concentrations of pure curcumin was not different from the vehicle control (Figure 33). The viabilities of Molt4 cells after treatment with 5, 10, and 15 µM were 93, 92, and 91%, respectively, while the vehicle control was 92%. Moreover, treatment with 5, 10, and 15 µM of pure curcumin decreased the WT1 mRNA level in a dose dependent manner by 6, 19, and 21%, respectively (Figure 34).

3.6.4 Effect of duration of pure curcumin treatments on *WT1* gene expression in Molt4 cell line

According to the results in section 3.6.2, pure curcumin had the strongest inhibitory effect on *WT1* gene expression in Molt4 cell. To evaluate its effect in a time dependent manner, the leukemic cell lines were treated with 10 µM of pure curcumin for 1, 2, and 3 days. For the vehicle control, the cells were incubated with 0.05% DMSO for 3 days. After incubation, the cells were examined for morphology and viability. Total RNA extraction (1 µg/reaction) and RT-PCR were carried out. The PCR products (15 µL) were electrophoresed and visualized using a scan densitometer, as described in sections 2.6 and 2.7. The experiment was done three times, and the WT1 mRNA levels were normalized by the GAPDH mRNA level. The morphology of Molt4 cells after treatment with pure curcumin was not different to the vehicle control cells (Figure 35). The cell viabilities of Molt4 cells in vehicle control group and after pure curcumin treatment for 1, 2, and 3 days were 92, 90, 89, and 88%, respectively. In addition, treatment with pure curcumin for all incubation times significantly decreased

the WT1 mRNA levels in a time dependent manner by 7, 11, and 16%, respectively (Figure 36).

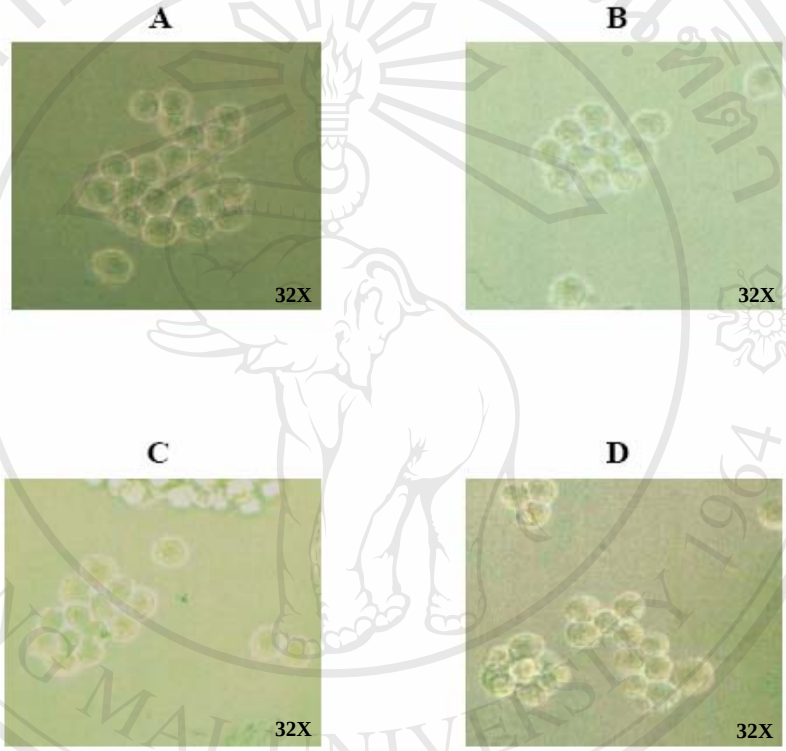

Figure 33. Molt4 cell morphology after pure curcumin treatment with 5, 10, and 15 µM. Cell morphology of Molt4 was examined after 48 h of treatment with (A) 0.05% DMSO, (B) 5 µM pure curcumin, (C) 10 µM pure curcumin, and (D) 15 µM pure curcumin.

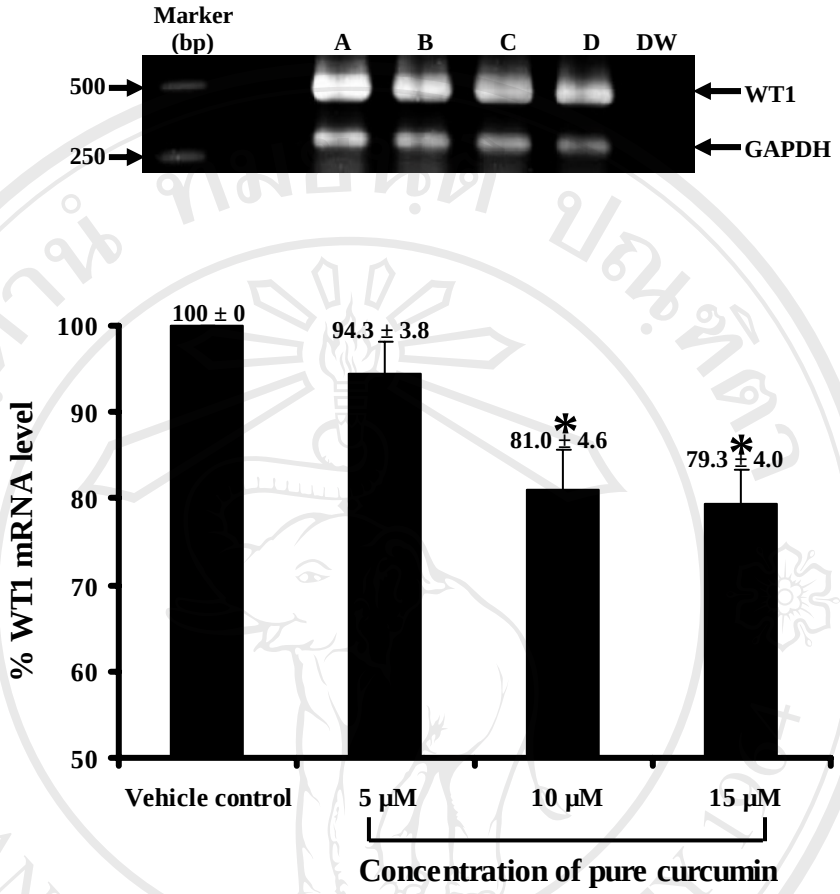

Figure 34. The WT1 mRNA levels in Molt4 cell line cultured in 5, 10, and 15 µM of pure curcumin for 2 days. The WT1 and GAPDH mRNA levels following treatment with (A) 0.05% DMSO and pure curcumin at a concentration (B) 5 µM, (C) 10 µM, and (D) 15 µM were determined in Molt4 cells after 2 days by RT-PCR. Fifteen microlitre of the PCR products were electrophoresed on 1% agarose gel. The bands were quantified using a scan densitometer. WT1 mRNA level was measured and normalized with GAPDH mRNA level. Distilled water (DW) was used as a negative control. Data are the mean value ± SD of three independent experiments. Asterisks (*****) denote values that were significantly different from the vehicle control ($p < 0.05$).

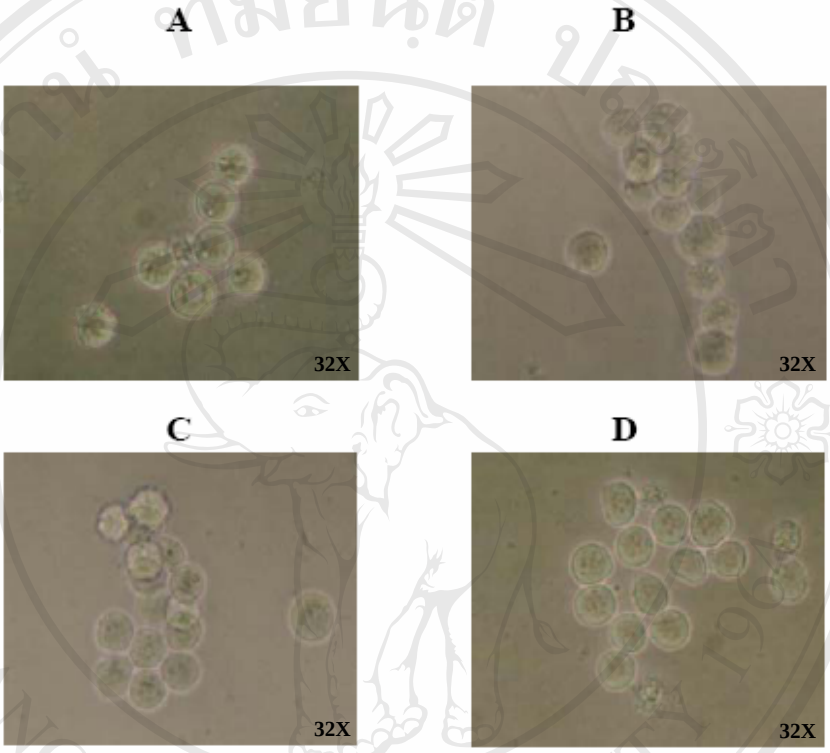

Figure 35. Molt4 cell morphology after treatment with 10 µM of pure curcumin for 1, 2, and 3 days. Cell morphology of Molt4 was examined after treatment with (A) 0.05% DMSO for 3 days and 10 µM pure curcumin for (B) 1 day, (C) 2 days, and (D) 3 days.

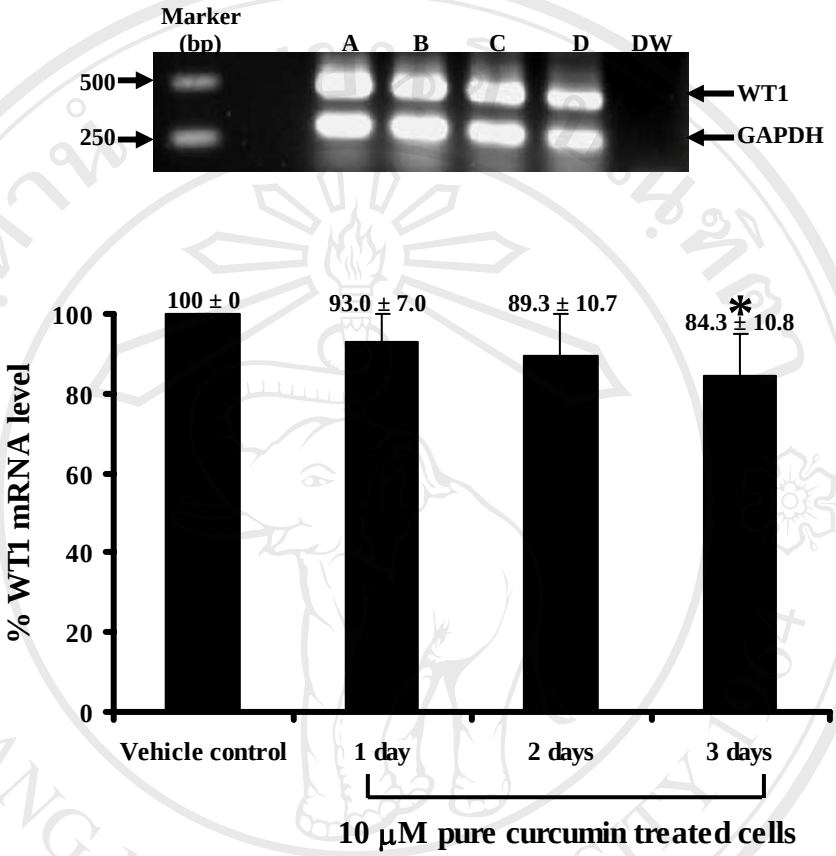

Figure 36. The WT1 mRNA levels in Molt4 cell line cultured in 10 μM of pure curcumin for 1, 2, and 3 days. The WT1 and GAPDH mRNA levels following treatment with (A) 0.05% DMSO for 3 days and 10 μM pure curcumin for (B) 1 day, (C) 2 days, and (D) 3 days were determined in Molt4 by RT-PCR. Fifteen microlitre of the PCR products were electrophoresed on 1% agarose gel. The bands were quantified using a scan densitometer. WT1 mRNA level was measured and normalized with GAPDH mRNA level. Distilled water (DW) was used as a negative control. Data are the mean value ± SD of three independent experiments. Asterisks (*****) denote values that were significantly different from the vehicle control ($p < 0.05$).

3.7 Level of WT1 protein in leukemic cell lines

To clarify whether the levels of WT1 protein in all leukemic cell lines were sufficient for detection after curcuminoids treatment, 4 types of untreated leukemic cell lines were grown in complete RPMI 1640 medium. After reaching 80% cell confluence, nuclear membrane protein extraction (100 µg/lane) and Western blot analysis were carried out to monitor the WT1 protein level, as described in sections 2.9.1 and 2.9.2. After WT1 detection, the nitrocellulose membrane was stripped and the GAPDH protein was detected in the same nitrocellulose membrane, as described in sections 2.9.3 and 2.9.4. The experiment was done three times. The WT1 proteins were detected in only K562 and Molt 4 cell lines, and not detected in U937 and HL-60. However, GAPDH protein levels were detected in all leukemic cell lines (figure 37). Thus the K562 and Molt4 cell lines were further investigated for the effect of curcuminoids derivatives on WT1 protein expression.

3.8 Effect of curcuminoids on WT1 protein level in K562 cell line

3.8.1 Effect of curcuminoids derivatives on WT1 protein level in K562 cell line

To study the effect of turmeric curcuminoid extracts on WT1 protein level in K562 cell line, the leukemic cell lines were cultured in RPMI 1640 medium with 0.05% DMSO as a vehicle control and 10 µM of each form of curcuminoids, including commercial grade curcuminoid mixture (Sigma-Aldrich), curcuminoid mixture, pure curcumin, demethoxycurcumin, and bisdemethoxycurcumin for 2 days. After treatment, the cell viability of K562 cells was determined. The cell viabilities after treatment with commercial grade curcuminoid mixture (Sigma-Aldrich), curcuminoid mixture, pure curcumin, demethoxycurcumin, bisdemethoxycurcumin, and vehicle control were 90, 89, 91, 89, 88, and 93%, respectively by the trypan blue exclusion method. A concentration of 10 µM of all curcuminoids derivatives did not produce toxic effects on K562 cells. Nuclear protein extraction (100 µg/lane) and Western blot analysis were performed to investigate the WT1 protein level, as described in section 2.9. After normalization with GAPDH protein level, WT1 protein expression was found to be decreased by 40, 22, 48, 13, and 42% in response to treatment with commercial grade curcuminoid mixture (Sigma-Aldrich), curcuminoid mixture, pure curcumin, demethoxycurcumin, and bisdemethoxycurcumin, respectively (Figure 38).

In addition, treatment with pure curcumin, bisdemethoxycurcumin, and commercial grade curcuminoid mixture clearly significantly decreased WT1 protein level when compared with the vehicle control. Moreover, pure curcumin treatment showed the strongest inhibitory effect on WT1 protein level in K562 cell line. This result was used for further study of the effect of pure curcumin on WT1 protein level by dose and time dependence.

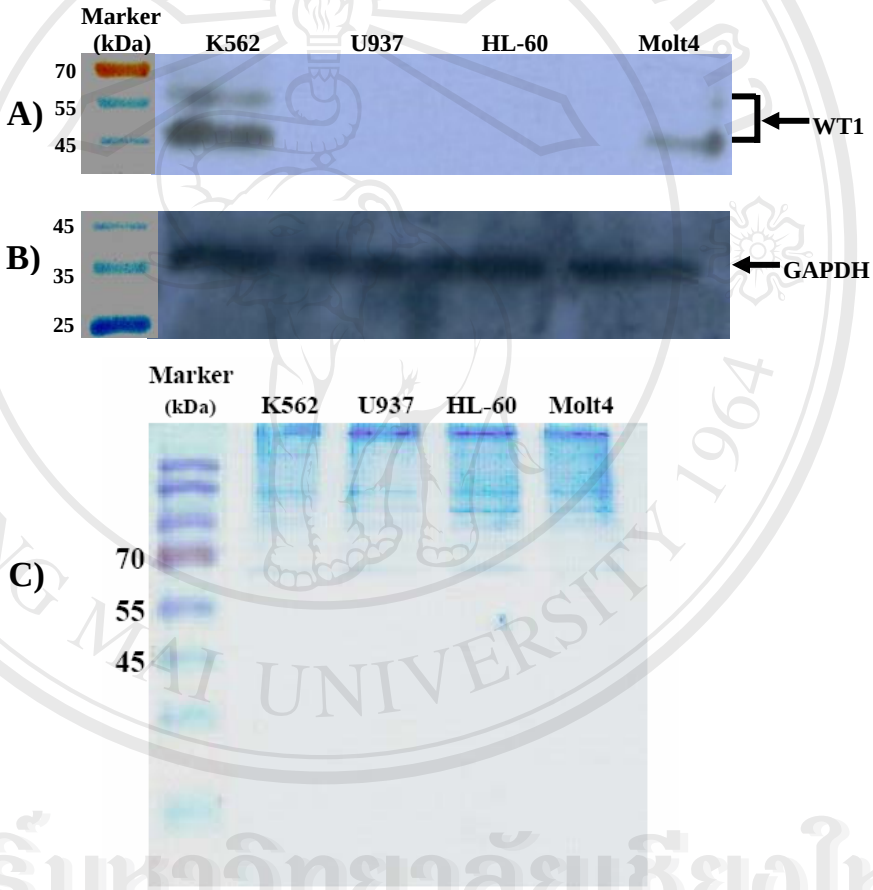

Figure 37. WT1 protein level in 4 types of leukemic cell line cultured in RPMI 1640 medium. The WT1 protein level was determined by Western blot analysis using rabbit polyclonal anti-WT1 antibody (WT1; C-19). The specific bands (A) were presented in only K562 and Molt4 cells. The level of GAPDH protein (B), and the Coomassie blue staining gel (C) were used as an internal control.

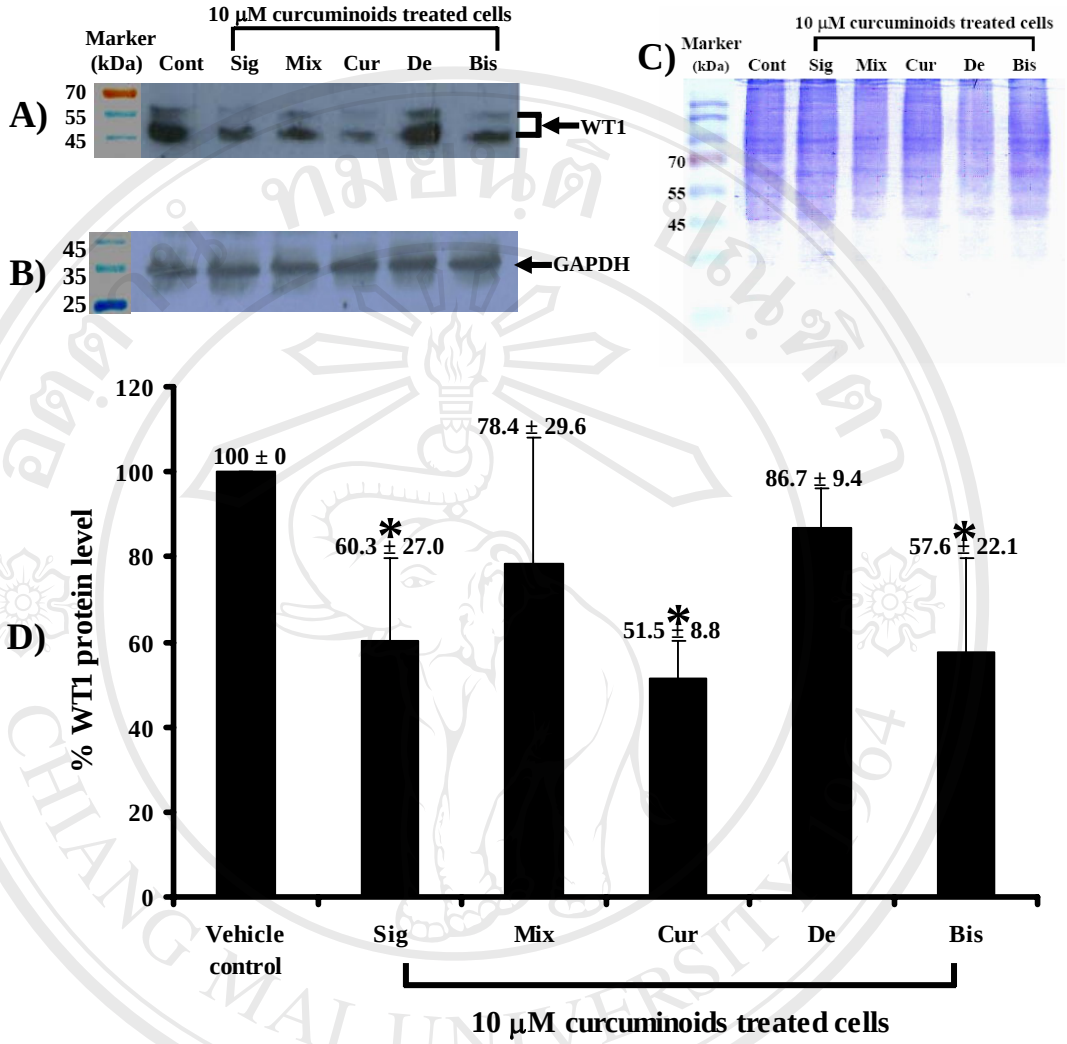

Figure 38. Analysis of the effect of curcuminoid extracts on WT1 protein level in K562 cell line. The WT1 and GAPDH protein levels following treatment with 0.05% DMSO (Cont) and 10 µM of commercial grade curcuminoid mixture (Sig), curcuminoid mixture (Mix), pure curcumin (Cur), demethoxycurcumin (De), and bisdemethoxycurcumin (Bis) were determined in K562 cells after 2 days by Western blot analysis. The bands (48 to 54 kDa for WT1 and 37 kDa for GAPDH were quantified using a scan densitometer. WT1 protein levels (A) were measured and normalized with GAPDH protein level (B). The Coomassie blue staining gel (C) was used as an internal control. Data (D) are the mean value ± standard deviation (SD) of three independent experiments. Asterisks (*) denote values that were significantly different from the vehicle control ($p < 0.05$).

3.8.2 Effect of concentrations of pure curcumin on WT1 protein level in K562 cell line

According to the results in section 3.8.1, pure curcumin had a strong inhibitory effect on WT1 protein level in K562 cells. In order to study its effect on WT1 protein level in a dose dependent manner, non-toxic doses of pure curcumin (5, 10, and 15 µM) were used in this experiment. K562 cell lines were cultured in RPMI 1640 medium with 0.05% DMSO (vehicle control) and each concentration of pure curcumin for 2 days. After incubation, the viability of K562 cells was determined. The viabilities of the cells after treatment with 5, 10 and, 15 µM of pure curcumin and 0.05% DMSO were 94, 92, 91, and 94%, respectively. Nuclear protein extraction (100 µg/lane) and Western blot analysis were performed to investigate the WT1 protein level, as described in section 2.9. After normalization with GAPDH protein level, WT1 expression decreased by 17, 37, and 81% in response to treatment with 5, 10, and 15 µM of pure curcumin, respectively (Figure 39). In addition, all concentrations of pure curcumin clearly significantly decreased WT1 protein level when compared with the level in the vehicle control.

3.8.3 Effect of duration of pure curcumin treatments on WT1 protein level in K562 cell line

To investigate the effect of pure curcumin on WT1 protein expression in a time dependent manner, K562 cell lines were cultured in RPMI 1640 medium with 0.05% DMSO and 10 µM of pure curcumin for 1, 2, and 3 days. Cell viability was determined by the trypan blue exclusion test. The cell viabilities after 005% DMSO and 10 µM of pure curcumin treatment for 1, 2, and 3 days were 95, 93, 91, and 89 %, respectively. Nuclear protein extraction (100 µg/lane) and Western blot analysis was used to investigate the level of WT1 protein expression, as described in section 2.9. After normalization with GAPDH protein level, WT1 protein levels decreased by 50.1, 91.6, and 99.2% in response to treatment with 10 µM of pure curcumin for 1, 2, and 3 days, respectively (Figure 40). Moreover, all of the incubation times significantly decreased WT1 protein level when compared with the level in the vehicle control.

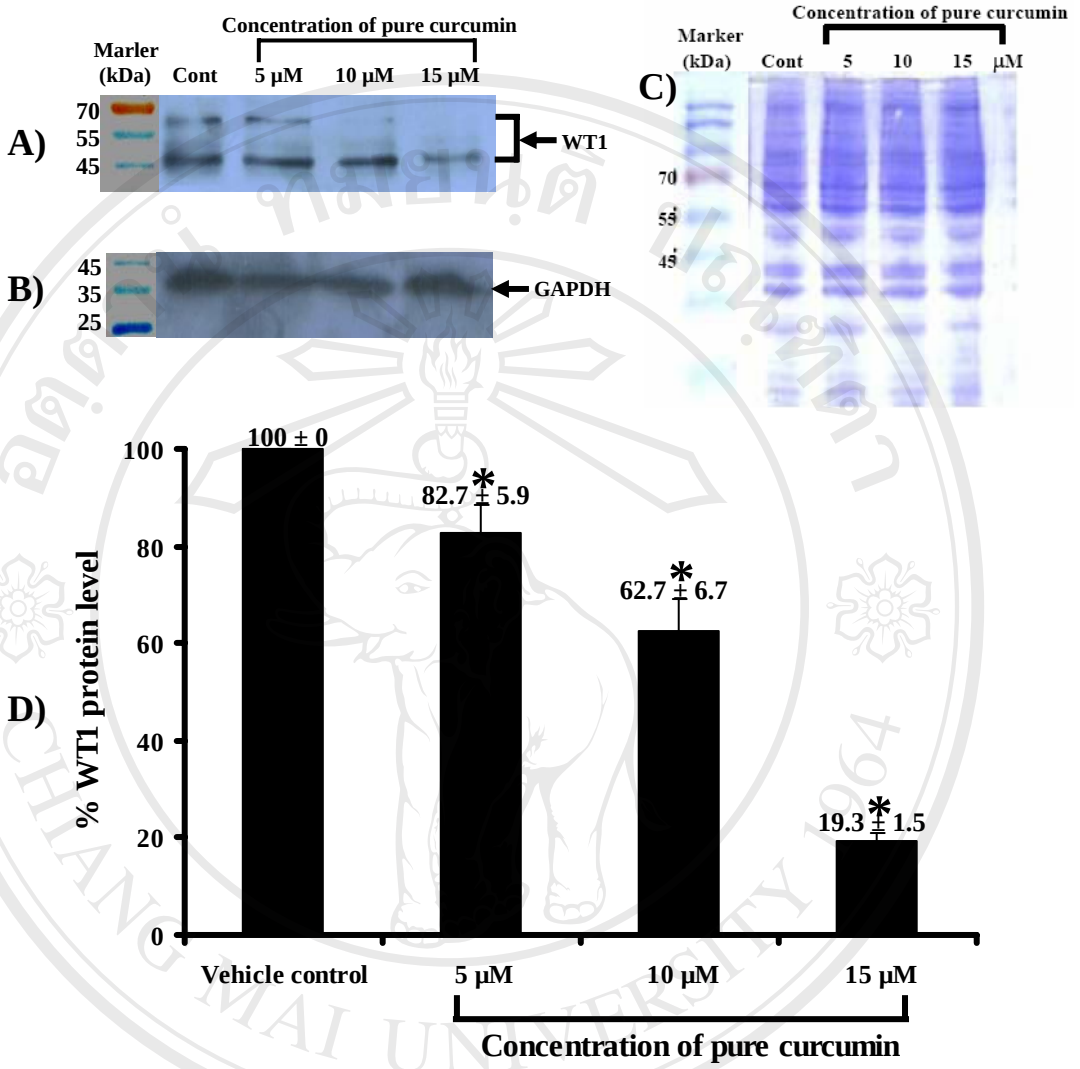

Figure 39. The WT1 protein level in K562 cell line cultured in 5, 10, and 15 µM of pure curcumin for 2 days. The WT1 and GAPDH protein levels following treatment with 0.05% DMSO (Cont) and pure curcumin at a concentration 5, 10, and 15 µM were determined in K562 cells after 2 days by Western blot analysis. The bands (48 to 54 kDa for WT1 and 37 kDa for GAPDH were quantified using a scan densitometer. WT1 protein levels (A) were measured and normalized with GAPDH protein level (B). The Coomassie blue staining gel (C) was used as an internal control. Data (D) are the mean value ± standard deviation (SD) of three independent experiments. Asterisks (*) denote values that were significantly different from the vehicle control ($p < 0.05$).

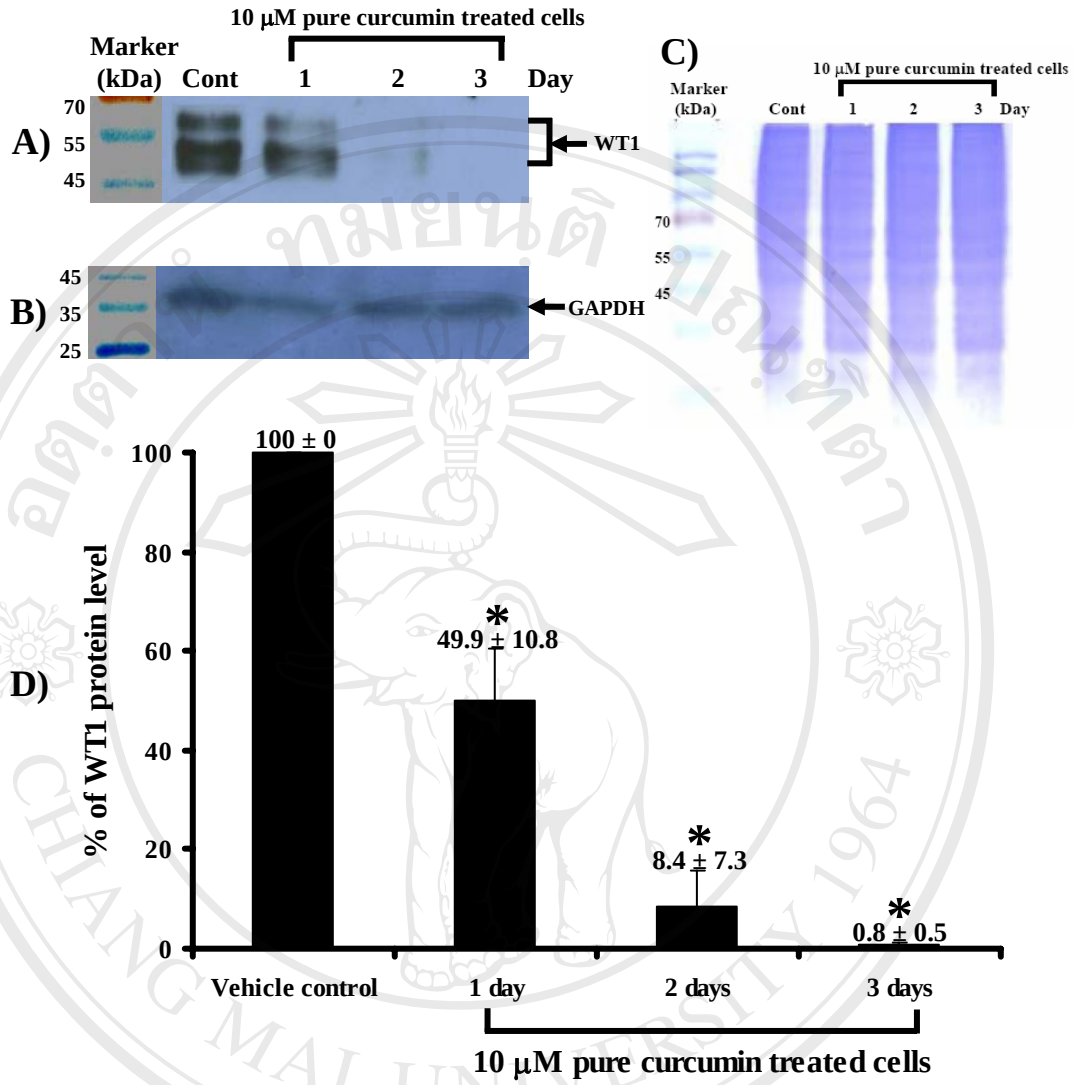

Figure 40. The WT1 protein level in K562 cell line cultured in 10 µM of pure curcumin for 1, 2, and 3 days. The WT1 and GAPDH protein levels following treatment with 0.05% DMSO (Cont) and 10 µM of pure curcumin for 1, 2, and 3 days were determined in K562 cells by Western blot analysis. The bands (48 to 54 kDa for WT1 and 37 kDa for GAPDH were quantified using a scan densitometer. WT1 protein levels (A) were measured and normalized with GAPDH protein level (B). The Coomassie blue staining gel (C) was used as an internal control. Data (D) are the mean value ± standard deviation (SD) of three independent experiments. Asterisks (*) denote values that were significantly different from the vehicle control ($p < 0.05$).

3.9 Effect of curcuminoids on WT1 protein level in Molt4 cell line

3.9.1 Effect of curcuminoids derivatives on WT1 protein level in Molt4 cell line

To study the effect of turmeric curcuminoid extracts on WT1 protein level in Molt4 cell line, the leukemic cell lines were cultured in RPMI 1640 medium with 0.05% DMSO as a vehicle control and 10 µM of each form of curcuminoids, including commercial grade curcuminoid mixture (Sigma-Aldrich), curcuminoid mixture, pure curcumin, demethoxycurcumin, and bisdemethoxycurcumin for 2 days. After treatment, the cell viability of Molt4 cells was determined. The cell viabilities after treatment with commercial grade curcuminoid mixture (Sigma-Aldrich), curcuminoid mixture, pure curcumin, demethoxycurcumin, bisdemethoxycurcumin, and vehicle control were 92, 91, 91, 92, 89, and 94%, respectively by the trypan blue exclusion method. A concentration of 10 µM of all curcuminoids derivatives did not produce toxic effects on Molt4 cells. Nuclear protein extraction (100 µg/lane) and Western blot analysis were performed to investigate the WT1 protein level, as described in section 2.9. After normalization with GAPDH protein level, WT1 protein expression was found to be decreased by 30, 26, 90, 57, and 68%in response to treatment with commercial grade curcuminoid mixture (Sigma-Aldrich), curcuminoid mixture, pure curcumin, demethoxycurcumin, and bisdemethoxycurcumin, respectively (Figure 41). In addition, treatment with pure curcumin, bisdemethoxycurcumin, demethoxycurcumin, and commercial grade curcuminoid mixture significantly decreased WT1 protein levels when compared with the vehicle control. Moreover, pure curcumin treatment showed the strongest inhibitory effect on WT1 protein level in Molt4 cell line. Pure curcumin was thus used for further study its effect on WT1 protein level by dose and time dependence.

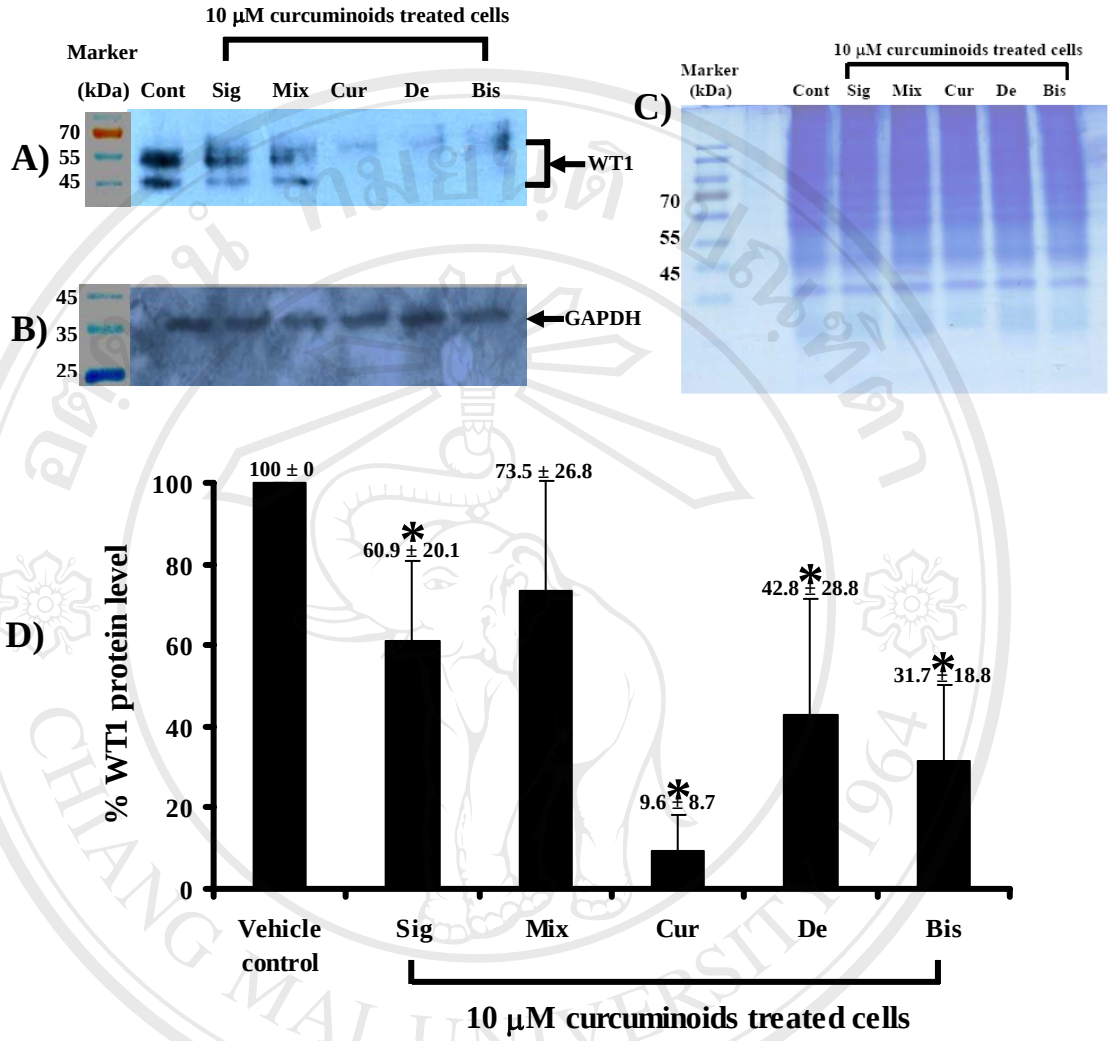

Figure 41. Analysis of the effect of curcuminoid extracts on WT1 protein level in Molt4 cell line. The WT1 and GAPDH protein levels following treatment with 0.05% DMSO (Cont) and 10 µM of commercial grade curcuminoid mixture (Sig), curcuminoid mixture (Mix), pure curcumin (Cur), demethoxycurcumin (De), and bisdemethoxycurcumin (Bis) were determined in Molt4 cells after 2 days by Western blot analysis. The bands (48 to 54 kDa for WT1 and 37 kDa for GAPDH were quantified using a scan densitometer. WT1 protein levels (A) were measured and normalized with GAPDH protein level (B). The Coomassie blue staining gel (C) was used as an internal control. Data (D) are the mean value ± standard deviation (SD) of three independent experiments. Asterisks (*) denote values that were significantly different from the vehicle control ($p < 0.05$).

3.9.2 Effect of concentrations of pure curcumin on WT1 protein level in Molt4 cell line

According to the results in section 3.9.1, pure curcumin has a strong inhibitory effect on WT1 protein level in Molt4 cells. In order to study its effect on WT1 protein level in dose dependent manner, non-toxic doses of pure curcumin (5, 10, and 15 µM) were used in this experiment. Molt4 cell lines were cultured in RPMI 1640 medium with 0.05% DMSO (vehicle control) and each concentration of pure curcumin for 2 days. After incubation, the viability of Molt4 cells was determined. The viabilities of the cells after treatment with 5, 10, and 15 µM of pure curcumin and 0.05% DMSO were 94, 96, 87, and 94%, respectively. Nuclear protein extraction (100 µg/lane) and Western blot analysis were performed to investigate the WT1 protein level, as described in section 2.9. After normalization with GAPDH protein level, WT1 expression was decreased by 56, 79, and 94% in response to treatment with 5, 10, and 15 µM of pure curcumin, respectively (Figure 42). In addition, all concentrations of pure curcumin significantly decreased WT1 protein level when compared with the level in the vehicle control.

3.9.3 Effect of duration of pure curcumin treatments on WT1 protein level in Molt4 cell line

To investigate the effect of pure curcumin on WT1 protein expression in a time dependent manner, Molt4 cell lines were cultured in RPMI 1640 medium with 0.05% DMSO and 10 µM of pure curcumin for 1, 2, and 3 days. Cell viability was determined by the trypan blue exclusion test. The cell viabilities after 005% DMSO and 10 µM of pure curcumin treatment for 1, 2, and 3 days were 90, 94, 90, and 88 %, respectively. Nuclear protein extraction (100 µg/lane) and Western blot analysis was used to investigate the level of WT1 protein expression, as described in section 2.9. After normalization with GAPDH protein level, WT1 protein levels were decreased by 53, 84, and 92% in response to treatment with 10 µM of pure curcumin for 1, 2, and 3 days, respectively (Figure 43). Moreover, all of the incubation times significantly decreased WT1 protein level when compared with the level in the vehicle control.

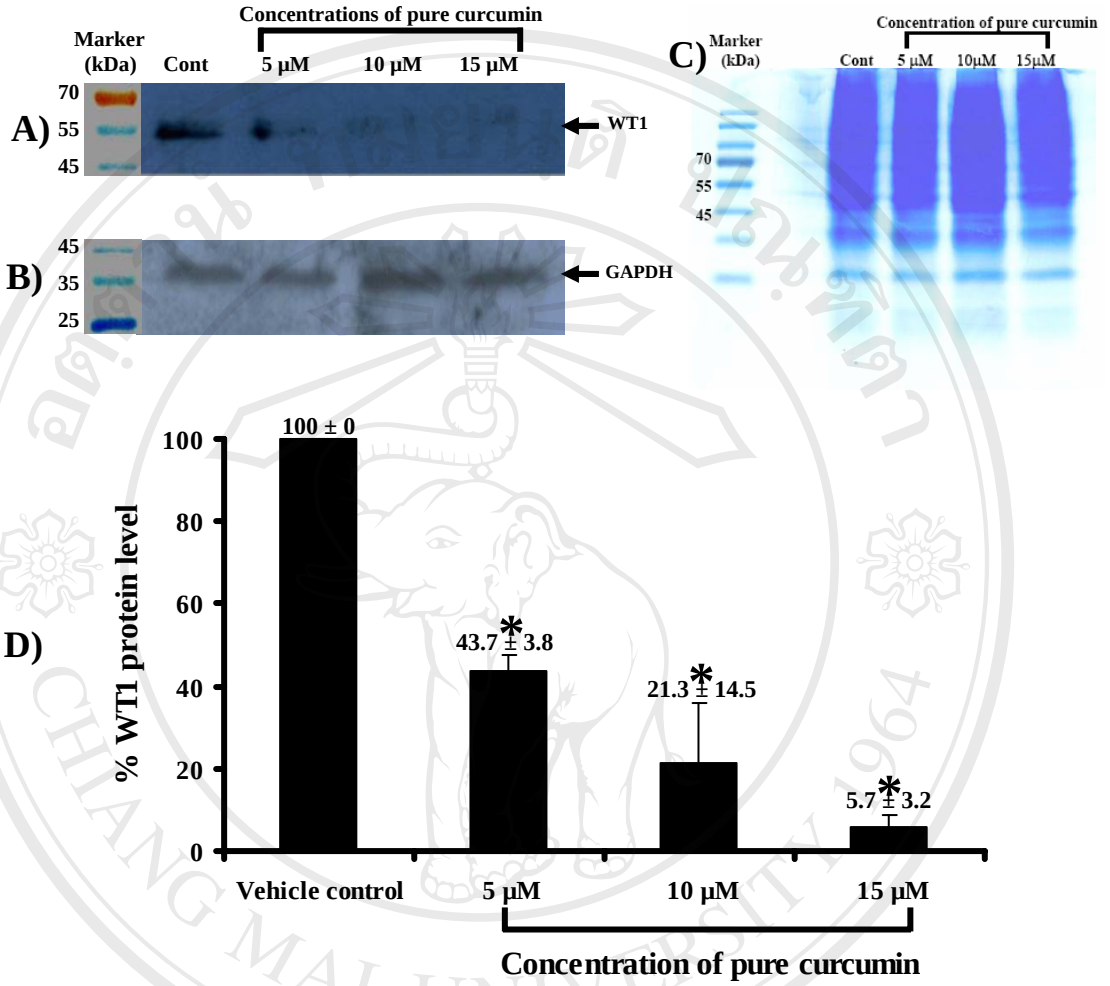

Figure 42. The WT1 protein level in Molt4 cell line cultured in 5, 10, and 15 µM of pure curcumin for 2 days. The WT1 and GAPDH protein levels following treatment with 0.05% DMSO (Cont) and pure curcumin at a concentration 5, 10, and 15 µM were determined in Molt4 cells after 2 days by Western blot analysis. The bands (48 to 54 kDa for WT1 and 37 kDa for GAPDH were quantified using a scan densitometer. WT1 protein levels (A) were measured and normalized with GAPDH protein level (B). The Coomassie blue staining gel (C) was used as an internal control. Data (D) are the mean value ± standard deviation (SD) of three independent experiments. Asterisks (*) denote values that were significantly different from the vehicle control ($p < 0.05$).

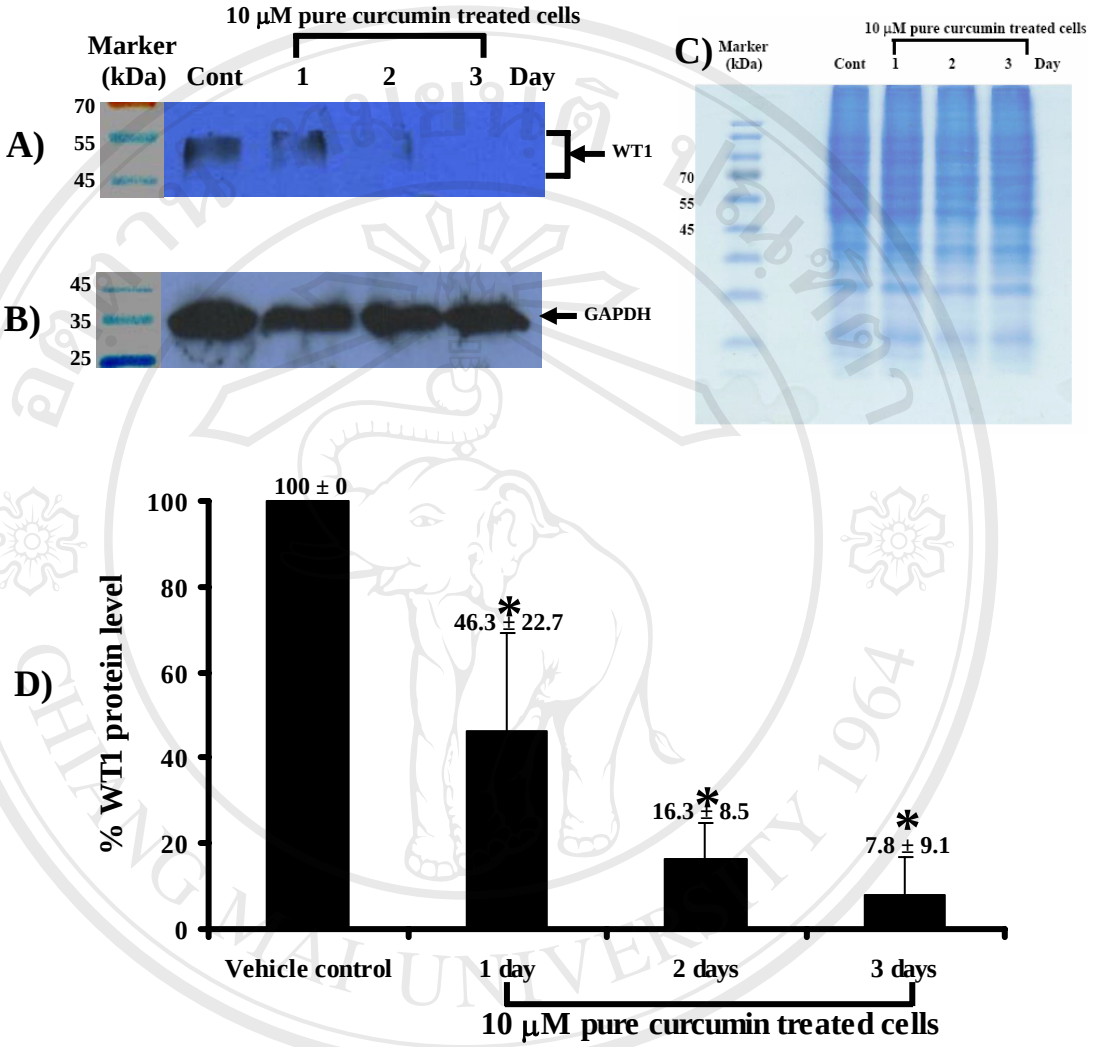

Figure 43. The WT1 protein level in Molt4 cell line cultured in 10 µM of pure pure curcumin for 1, 2, and 3 days. The WT1 and GAPDH protein levels following treatment with 0.05% DMSO (Cont) and 10 µM of pure curcumin for 1, 2, and 3 days were determined in Molt4 cells by Western blot analysis. The bands (48 to 54 kDa for WT1 and 37 kDa for GAPDH were quantified using a scan densitometer. WT1 protein levels (A) were measured and normalized with GAPDH protein level (B). The Coomassie blue staining gel (C) was used as an internal control. Data (D) are the mean value ± standard deviation (SD) of three independent experiments. Asterisks (*) denote values that were significantly different from the vehicle control ($p < 0.05$).