CHAPTER III

RESULTS

3.1 Cytotoxicity of green tea flavonoids in KB-V1 and KB-3-1 cell lines.

To examine whether exposure of catechin, EC, ECG, EGC and EGCG affects the viability of cells, multidrug resistant KB-V1 and its wild type KB-3-1 cells were exposed to various concentrations of green tea flavonoids for 48 h and cell viability was determined by MTT assay as described in Section 2.4. As shown in Figure 12 and 13, catechin and ECG were non toxic to the drug resistant (KB-V1) and drug sensitive (KB-3-1) cells but EC, EGC and EGCG were equally toxic to both cell lines in a dose dependent manner. Non toxic-concentrations (IC_{20}) leading to 80% cell survival in KB-V1 and KB-3-1 were 152.6 ± 5.0 and 153.7 ± 7.3 μM for EC, 142.4 ± 5.9 and 108.8 ± 5.0 μM for EGC and 101 ± 9.5 and 104.4 ± 3.5 μM for EGCG respectively. The IC_{20} and IC_{50} values of green tea flavonoids for KB-V1 and KB-3-1 are shown in Table 9 and 11. The non cytotoxic concentrations (IC_{20}) of all green tea flavonoids were used for MDR phenotype study (Section 3.4)

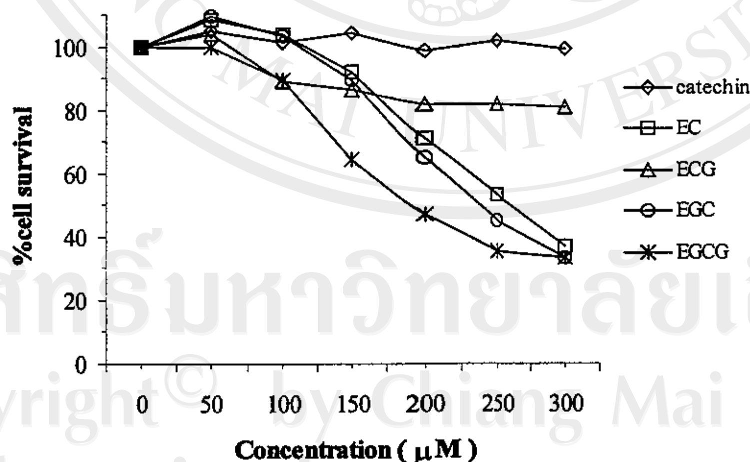

Figure 12. Cytotoxicity of green tea flavonoids in KB-V1 cells. Cells ($3x10^3$ cells/well), in 200 μl medium were grown in the presence of various concentrations of green tea flavonoids (catechin, EC, ECG, EGC and EGCG) for 48 h. The cell viability was determined by MTT assay. Each point represents the mean value of three independent experiments performed in triplicate.

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Table 9. Cytotoxicity of green tea flavonoids in KB-V1 cells. The data shown in figure12 were represented as mean values ± standard deviation of three independent experiments performed in triplicate.

Concentration (μM)	Cell survival (% of control)				
	catechin	EC	ECG	EGC	EGCG
0 (vehicle control)	100 ± 0	100 ± 0	100 ± 0	100 ± 0	100 ± 0
50	105 ± 2	108 ± 8	104 ± 2	110 ± 7	100 ± 4
100	101 ± 2	104 ± 11	89 ± 9	103 ± 1	90 ± 9
150	104 ± 4	92 ± 10	86 ± 8	89 ± 4	64 ± 2
200	99 ± 5	71 ± 9	82 ± 3	65 ± 3	47 ± 1
250	102 ± 5	53 ± 6	82 ± 3	45 ± 4	35 ± 1
300	99 ± 5	37 ± 8	81 ± 2	33 ± 4	33 ± 1

Table 10. IC_{20} and IC_{50} values of green tea flavonoids on cytotoxicity of KB-V1 cells. The data represent the mean values ± standard deviation of three independent experiments performed in triplicate.

Green tea flavonoids	IC_{20} Values (μM)	IC_{50} Values (μM)
Catechin	>300	>300
EC	152.6 ±5.0	280.9 ± 5.9
ECG	>300	>300
EGC	142.4 ± 5.9	257 ± 0.2
EGCG	101.0 ± 9.5	214.3 ± 8.9

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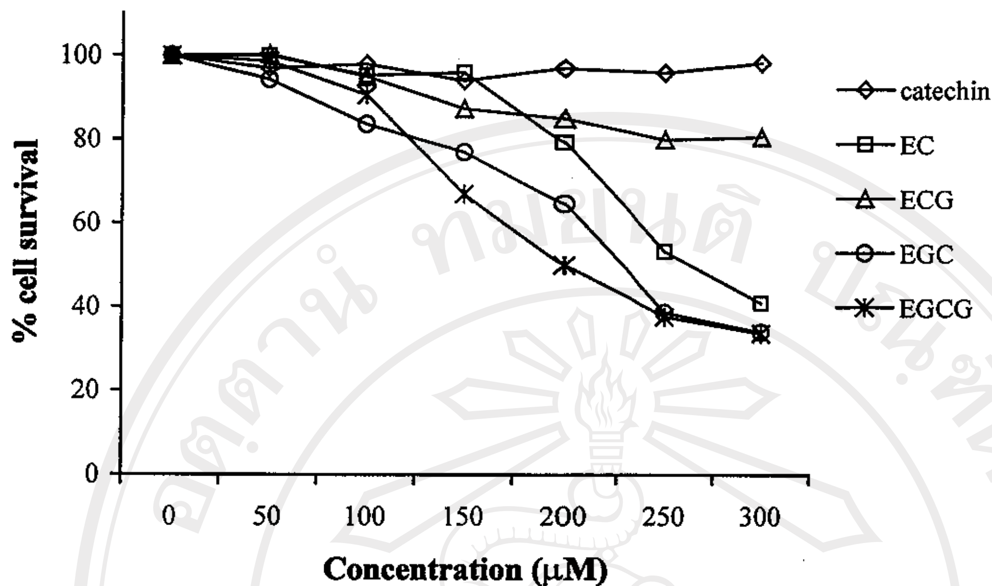

Figure 13. Cytotoxicity of green tea flavonoids in KB-3-1 cells. Cells ($3x10^3$ cells/well), in 200 μl medium were grown in the presence of various concentrations of green tea flavonoids (catechin, EC, ECG, EGC and EGCG) for 48 h. The cell viability was determined by MTT assay. Each point represents the mean value of three independent experiments performed in triplicate.

Table 11. Cytotoxicity of green tea flavonoids in KB-3-1 cells. The data shown in Figure13 were represented as mean values ± standard deviation of three independent experiments performed in triplicate.

Concentration (μM)	Cell survival (% of control)				
	catechin	EC	ECG	EGC	EGCG
0 (vehicle control)	100 ± 0	100 ± 0	100 ± 0	100 ± 0	100 ± 0
50	97 ± 3	100 ± 0	100 ± 2	94 ± 5	99 ± 3
100	98 ± 5	95 ± 2	95 ± 3	84 ± 4	91 ± 2
150	94 ± 3	96 ± 2	87 ± 5	77 ± 2	67 ± 4
200	97 ± 5	79 ± 9	85 ± 2	65 ± 5	50 ± 5
250	96 ± 2	53 ± 2	80 ± 1	39 ± 1	38 ± 2
300	98 ± 6	41 ± 4	81 ± 2	34 ± 3	34 ± 3

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Table 12. IC_{20} and IC_{50} values of green tea flavonoids on cytotoxicity of KB-3-1 cells. The data represent the mean values ± standard deviation of three independent experiments performed in triplicate.

Green tea flavonoids	IC_{20} Values (μM)	IC_{50} Values (μM)
Catechin	>300	>300
EC	153.7 ± 7.3	280.9 ± 5.9
ECG	>300	>300
EGC	108.8 ± 5	236.77 ± 13.8
EGCG	104.4 ± 3.5	220.6 ± 3.4

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3.2 Effect of green tea flavonoids on Pgp mediated drug transport

3.2.1 Effect of green tea flavonoids on Rh123 accumulation and efflux

To study the effect of green tea flavonoids on Rh123 accumulation and efflux, the activity of Pgp was assessed by measuring the intracellular retention of Rh123 fluorescence in Pgp expressing KB-V1 cells compared with its vehicle control (0.4% DMSO). Flow cytometry was carried out to investigate the amount of intracellular Rh123 in both accumulation and efflux experiments. In the presence of catechin, EC and EGC during the loading period of Rh123 for 60 min, KB-V1 accumulated substantially the same amount of Rh123 compared to the vehicle control. In Rh123 efflux studies, treatment of catechin, EC and EGC did not cause significant effect on Rh123 retention in KB-V1 cells (Figure14-16 and Table13 and14).

The treatment with EGCG at various concentrations increased the accumulation of Rh123 in KB-V1 cells. The intracellular Rh123 increased as a dose dependent manner and was statistically significant at 250-300 μM when compared with the vehicle control. In efflux studies, the Rh123 retention in KB-V1 cells after allowing 30 min for fluorescence dye to efflux, demonstrated that EGCG appeared to prevent Rh123 efflux and maintain Rh123 in the cells with a significant increase in Rh123 retention at 250 and 300 μM (Figure 17 and Table 13 and 14). EGCG did not cause significant effect on Rh123 accumulation and efflux in the KB-3-1 cells

The treatment of ECG had an effect similar to that observed by EGCG. ECG caused slightly but no significantly increase of Rh123 accumulation in KB-V1 cells. At concentrations of 250 and 300 μM, ECG significantly increased the retention of Rh123 in KB-V1 cells. Treatment of ECG in KB-3-1 cells did not cause significant increase of Rh123 accumulation and efflux (Figure 18and Table 13 and 14).

Verapamil at 30 μM was used as a positive control in this study and the data showed that it strongly increase Rh123 accumulation and retention in KB-V1 cells whereas it did not cause significant increase Rh123 accumulation and retention in KB-3-1 cells (Table 13 and 14).

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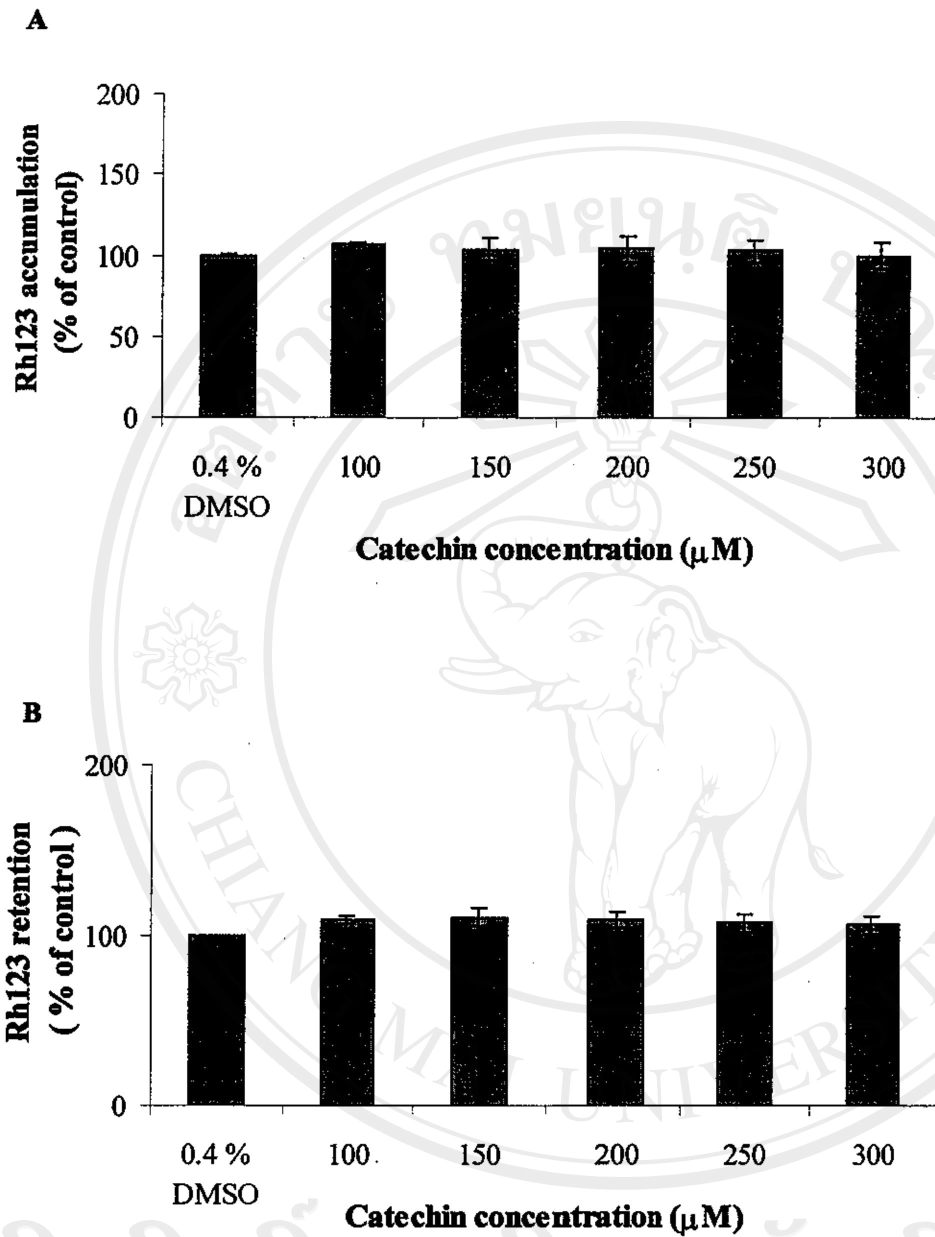

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Figure 14. Effect of catechin on Rh123 accumulation (A) and efflux (B) in Pgp expressing KB-V1 cell line. The amount of intracellular Rh123 fluorescence was determined by flow cytometry. Each point represented mean ± standard deviation of three independent experiments performed in triplicate.

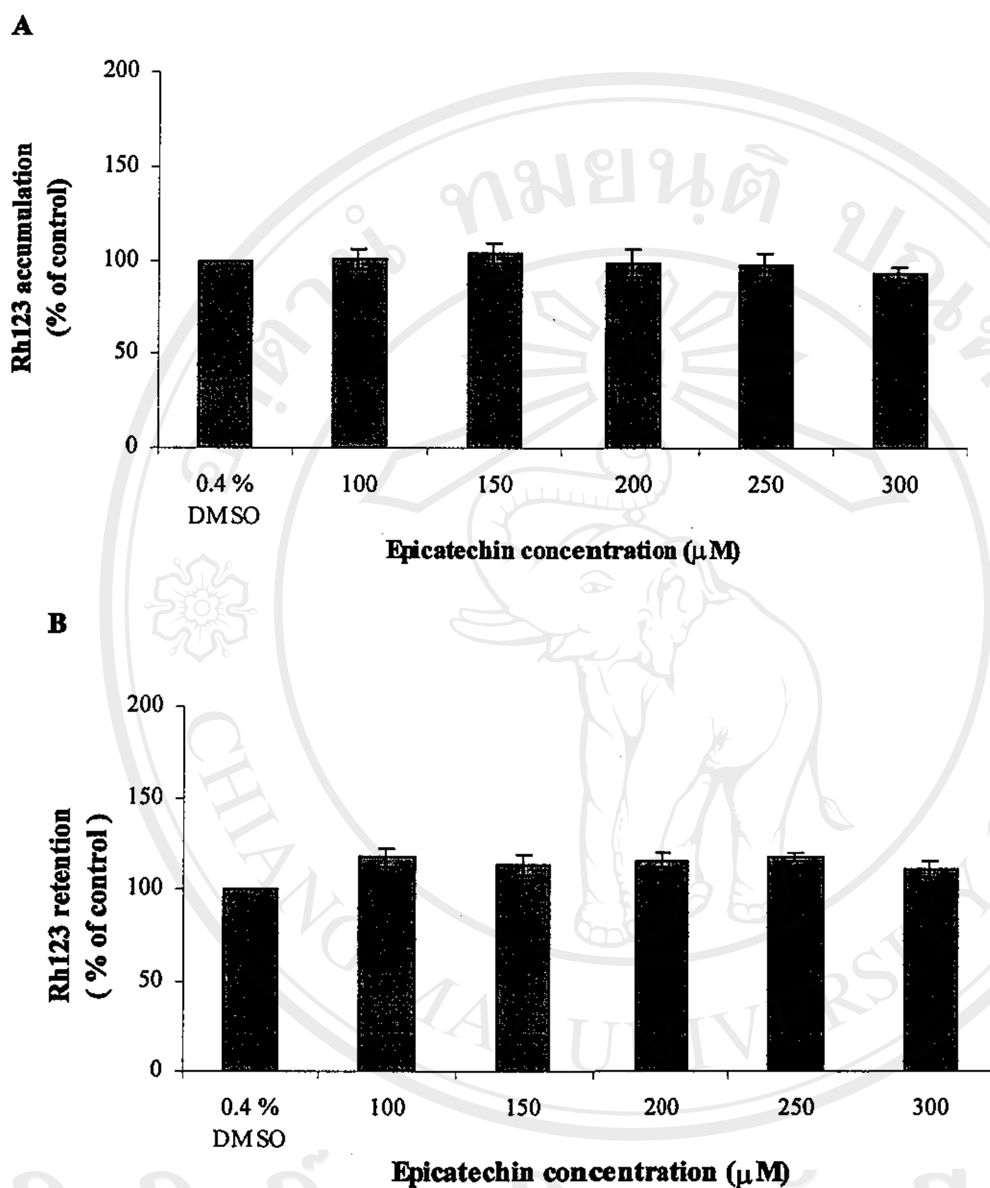

Figure 15. Effect of EC on Rh123 accumulation (A) and efflux (B) in Pgp expressing KB-V1 cell line. The amount of intracellular Rh123 fluorescence was determined by flow cytometry. Each point represented mean ± standard deviation of three independent experiments performed in triplicate.

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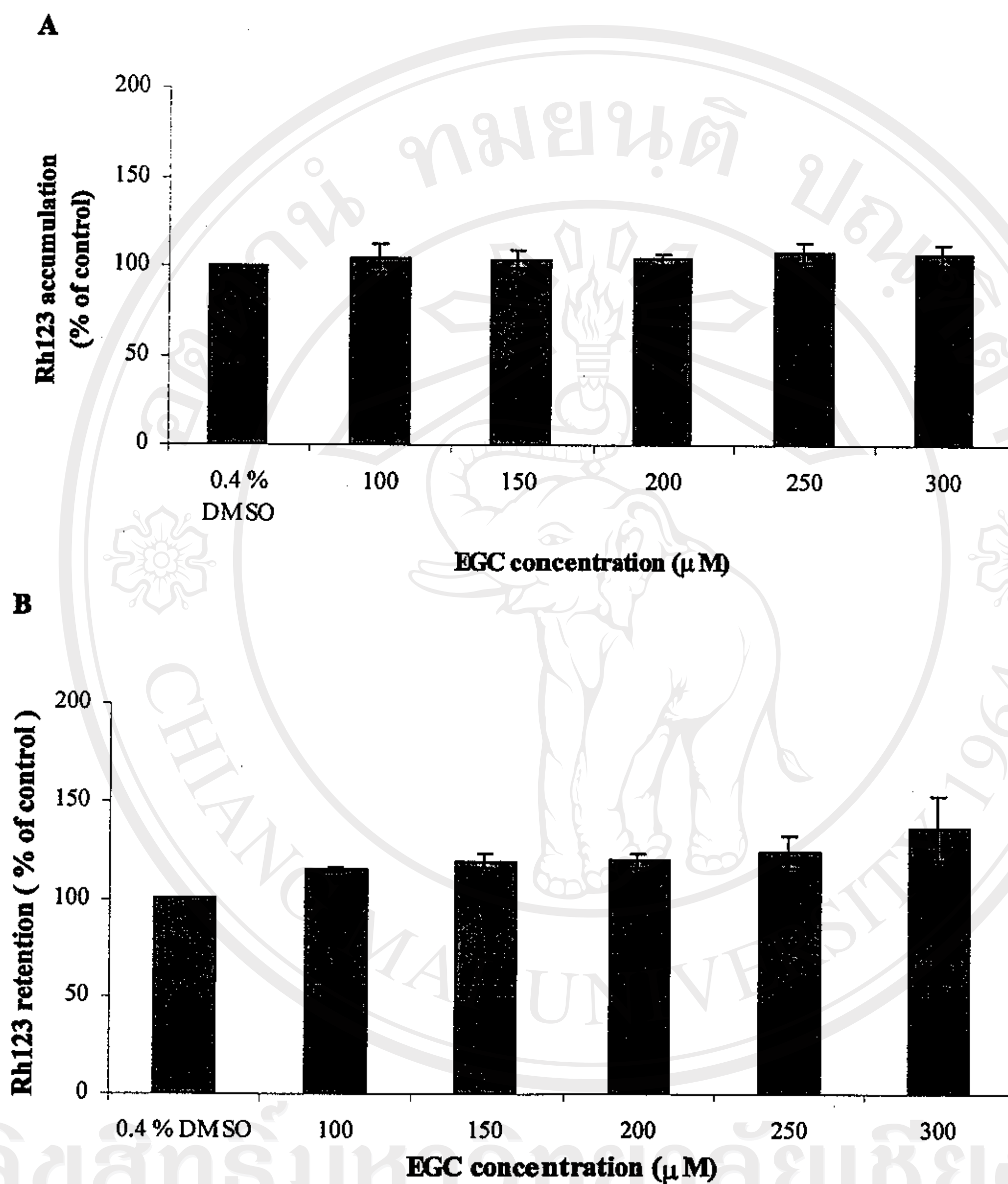

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Figure 16. Effect of EGC on Rh123 accumulation (A) and efflux (B) in Pgp expressing KB-V1 cell line. The amount of intracellular Rh123 fluorescence was determined by flow cytometry. Each point represented mean ± standard deviation of three independent experiments performed in triplicate.

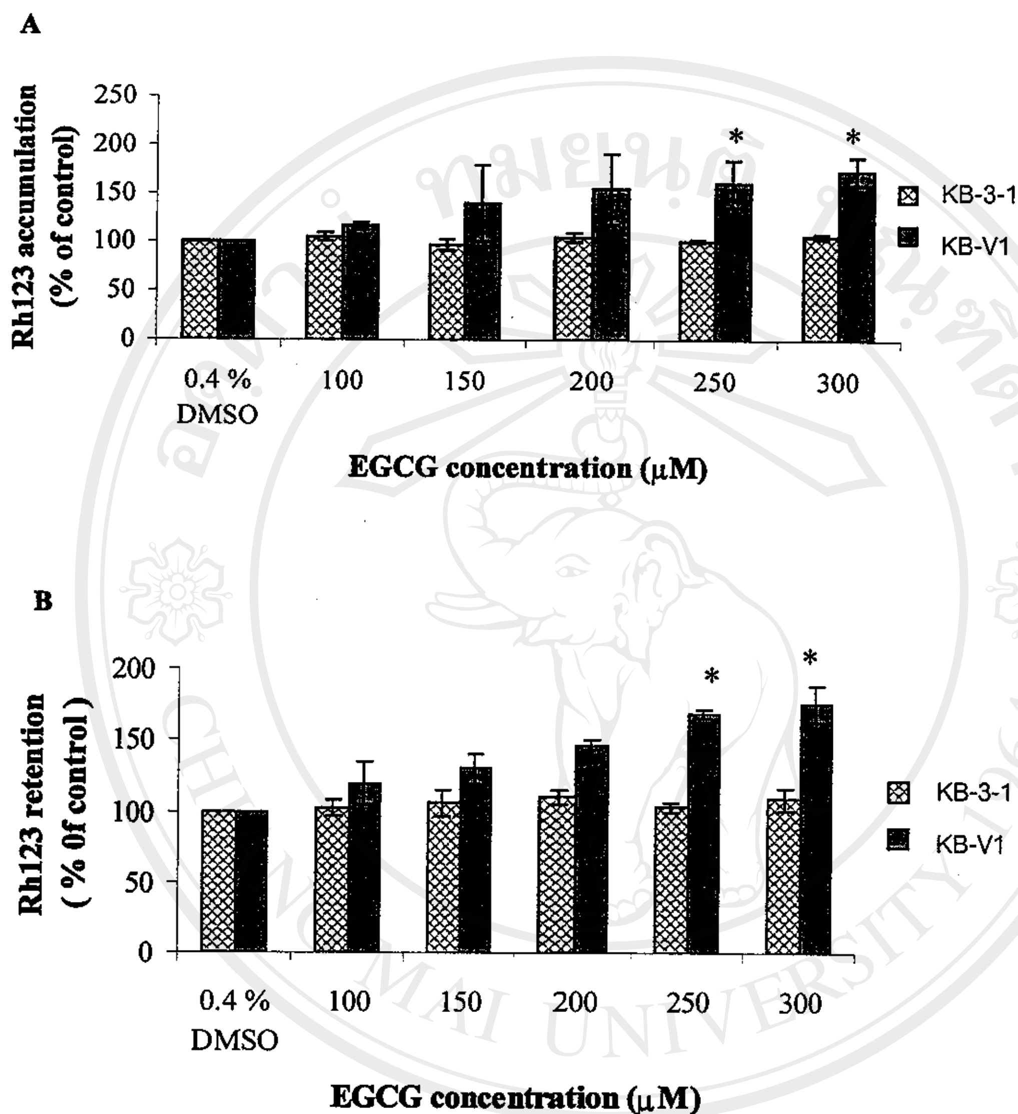

Figure 17. Effect of EGCG on Rh123 accumulation (A) and efflux (B) in Pgp expressing KB-V1 and KB-3-1 cell lines. The amount of intracellular Rh123 fluorescence was determined by flow cytometry. Each point represented mean ± standard deviation of three independent experiments performed in triplicate. Significant difference from untreated control was indicated by $p<0.05$ (*)

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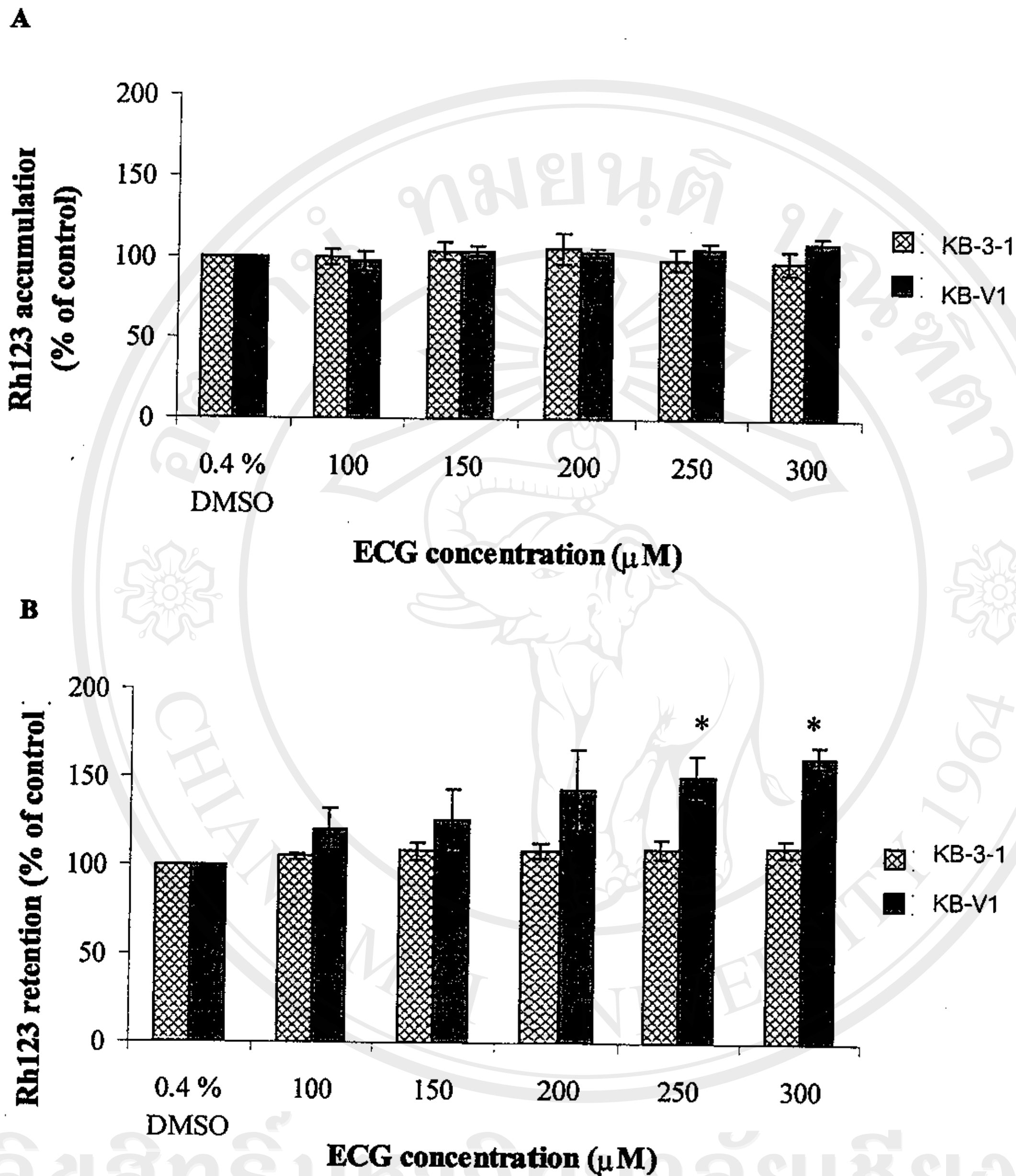

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Figure 18. Effect of ECG on Rh123 accumulation (A) and efflux (B) in Pgp expressing KB-V1 and KB-3-1 cell lines. The amount of intracellular Rh123 fluorescence was determined by flow cytometry. Each point represented mean ± standard deviation of three independent experiments performed in triplicate. Significant difference from untreated control was indicated by $p<0.05$ (*)

Table 13. Effect of green tea flavonoids on Rh123 accumulation in KB-V1 and KB-3-1 cells. The data showed in Figure 14-18 represented mean ± standard deviation of three independent experiments performed in triplicate.

Treatment	Concentration (μM)	Rh123 accumulation (% of control)	
		KB-V1	KB-3-1
catechin	0	100 ± 0.0	-
	100	107 ± 1.1	-
	150	104 ± 6.6	-
	200	104 ± 7.7	-
	250	103 ± 7.2	-
	300	100 ± 8.2	-
Verapamil	30	290 ± 52.1**	-
EC	0	100 ± 0.0	-
	100	100 ± 5.3	-
	150	104 ± 5.5	-
	200	98 ± 7.4	-
	250	97 ± 6.4	-
	300	93 ± 2.9	-
Verapamil	30	211 ± 42.4**	-
EGC	0	100 ± 00	-
	100	105 ± 7.6	-
	150	104 ± 5.3	-
	200	104 ± 3.0	-
	250	107 ± 5.6	-
	300	107 ± 5.8	-
Verapamil	30	238 ± 62.9**	-
EGCG	0	100 ± 0.0	100 ± 0.0
	100	117 ± 3.6	105 ± 4.2
	150	140 3± 9.3	97 ± 5.6
	200	155 3± 6.7	105 ± 4.5
	250	161 ± 24.1*	101 ± 1.7
	300	174 ± 14.0*	106 ± 2.6
Verapamil	30	387 ± 26.9**	108 ± 3.5
ECG	0	100 ± 0.0	100 ± 00
	100	98 ± 5.8	100 ± 4.8
	150	104 ± 3.0	106 ± 5.5
	200	103 ± 2.3	106 ± 9.8
	250	106 ± 3.3	99 ± 6.3
	300	109 ± 3.6	98 ± 7.4
Verapamil	30	216 ± 72.6**	102 ± 3.6

Significant difference from untreated control was indicated by $p<0.05$ (*) and $p<0.01$ (**)

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Table 14. Effect of green tea flavonoids on Rh123 efflux in KB-V1 and KB-3-1 cells. The data showed in Figure14-18 represented mean ± standard deviation of three independent experiments performed in triplicate.

Treatment	Concentration (μM)	Rh123 retention (% of control)	
		KB-V1	KB-3-1
catechin	0	100 ± 0.1	-
	100	109 ± 2.7	-
	150	111 ± 5.6	-
	200	109 ± 4.2	-
	250	108 ± 5.2	-
	300	107 ± 5.3	-
Verapamil	30	303 ± 79.0**	-
EC	0	100 ± 0.1	-
	100	117 ± 4.6	-
	150	114 ± 5.6	-
	200	116 ± 4.0	-
	250	118 ± 2.4	-
	300	111 ± 4.4	-
Verapamil	30	252 ± 62.4**	-
EGC	0	100 ± 0.1	-
	100	115 ± 1.2	-
	150	119 ± 3.8	-
	200	120 ± 3.6	-
	250	124 ± 3.6	-
	300	136 ± 16.5	-
Verapamil	30	277 ± 39.8**	-
EGCG	0	100 ± 0.0	100 ± 0.0
	100	120 ± 14.7	102 ± 5.4
	150	130 ± 10.8	106 ± 9.1
	200	146 ± 3.5	110 ± 4.5
	250	169 ± 3.3**	103 ± 3.2
	300	176 ± 13.1**	109 ± 7.8
Verapamil	30	332 ± 54.9**	105 ± 7.4
ECG	0	100 ± 0.1	100 ± 0.2
	100	121 ± 11.8	105 ± 1.9
	150	126 ± 17.7	108 ± 4.8
	200	143 ± 23.1	108 ± 4.6
	250	151 ± 12.7**	110 ± 5.1
	300	162 ± 5.6**	111 ± 4.8
Verapamil	30	267 ± 82.9**	110 ± 6.5

Significant difference from untreated control was indicated by $p<0.05$ (*) and $p<0.01$ (**)

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3.2.2 Effect of green tea flavonoids on ^{3}H-vinblastine accumulation and efflux.

To confirm the effect of green tea flavonoids on Pgp function, the activity of Pgp was assessed by determining radioisotope labeled drug, ^{3}H-vinblastine in the accumulation and efflux experiments. This method shows the actual vinblastine transport in the intact cells. Co-incubation of ^{3}H-vinblastine with catechin, EC and EGC for 60 min did not cause significant increase of ^{3}H-vinblastine accumulation in KB-V1 cell when compared with the vehicle control (Figure 19A, 20A and 21A and Table 15). In ^{3}H-vinblastine efflux studies, the cells were pre-incubated for 1 h with Pgp inhibitor, 10 μM CsA in order to load the ^{3}H-vinblastine into the cells, providing an equal amount of the intracellular ^{3}H-vinblastine in each treatment group, then the various concentrations of green tea flavonoids were added and further incubated for 15 min after removing the inhibitor containing medium. The ^{3}H-vinblastine retention was then determined and compared to the vehicle control. The treatment of catechin, EC and EGC did not cause significant effect on ^{3}H-vinblastine retention in KB-V1 cells (Figure 19B, 20B and 21B and Table 16).

The treatment with EGCG at various concentrations increased the accumulation of ^{3}H-vinblastine in KB-V1 cells. The intracellular ^{3}H-vinblastine increased dose dependently and caused significant increase at 200-300 μM when compared with the vehicle control (Figure 22A and Table 14). In efflux studies, the ^{3}H-vinblastine retention in KB-V1 cells demonstrated that EGCG appeared to prevent ^{3}H-vinblastine efflux with a significant increase at 300 μM in ^{3}H-vinblastine retention, compared to the vehicle control (Figure 22B and Table 16). The treatment of KB-3-1 cells with EGCG did not cause significant increase of ^{3}H-vinblastine accumulation and retention. The treatment of ECG showed similar effect to that observed by EGCG. ECG significantly increased accumulation of ^{3}H-vinblastine in KB-V1 cells at 200-300 μM. At concentration of 300 μM, ECG significantly increased the retention of ^{3}H-vinblastine in KB-V1 cells. The treatment of KB-3-1 cells with ECG did not cause significant increase of ^{3}H-vinblastine accumulation and retention (Figure 23 and Table 15 and 16).

Verapamil at 30 μM was used as a positive control in this study and the data showed that it strongly increased ^{3}H-vinblastine accumulation and retention in KB-V1 cells whereas it did not cause significant increase ^{3}H-vinblastine accumulation and retention in KB-3-1 cells (Table 15 and 16).

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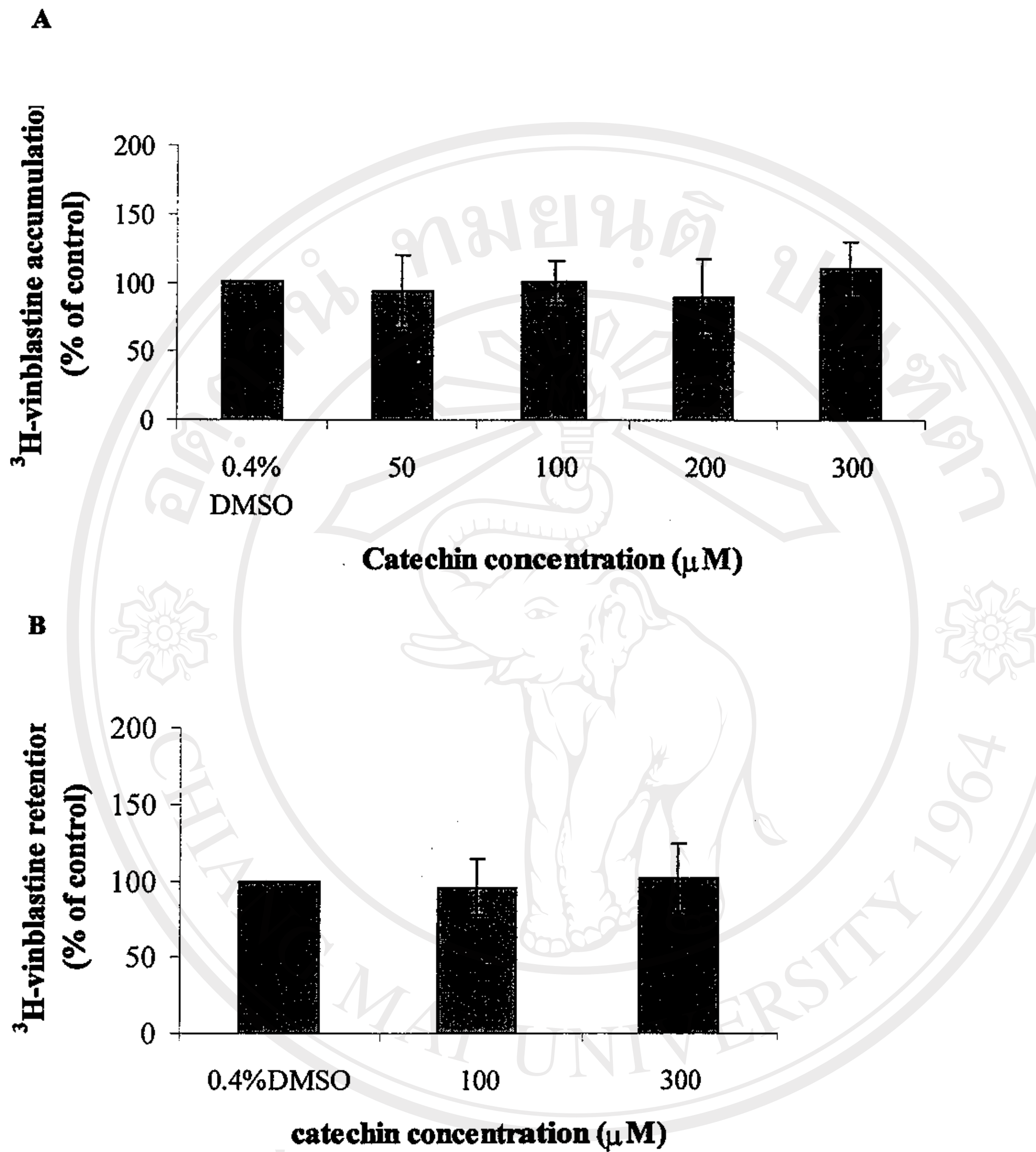

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Figure 19. Effect of catechin on ³H-vinblastine accumulation (A) and efflux (B) in KB-V1 cell line. The amount of intracellular radioactivity was measured by the β counter. Each point represented the mean value of three times independent experiments performed in triplicate.

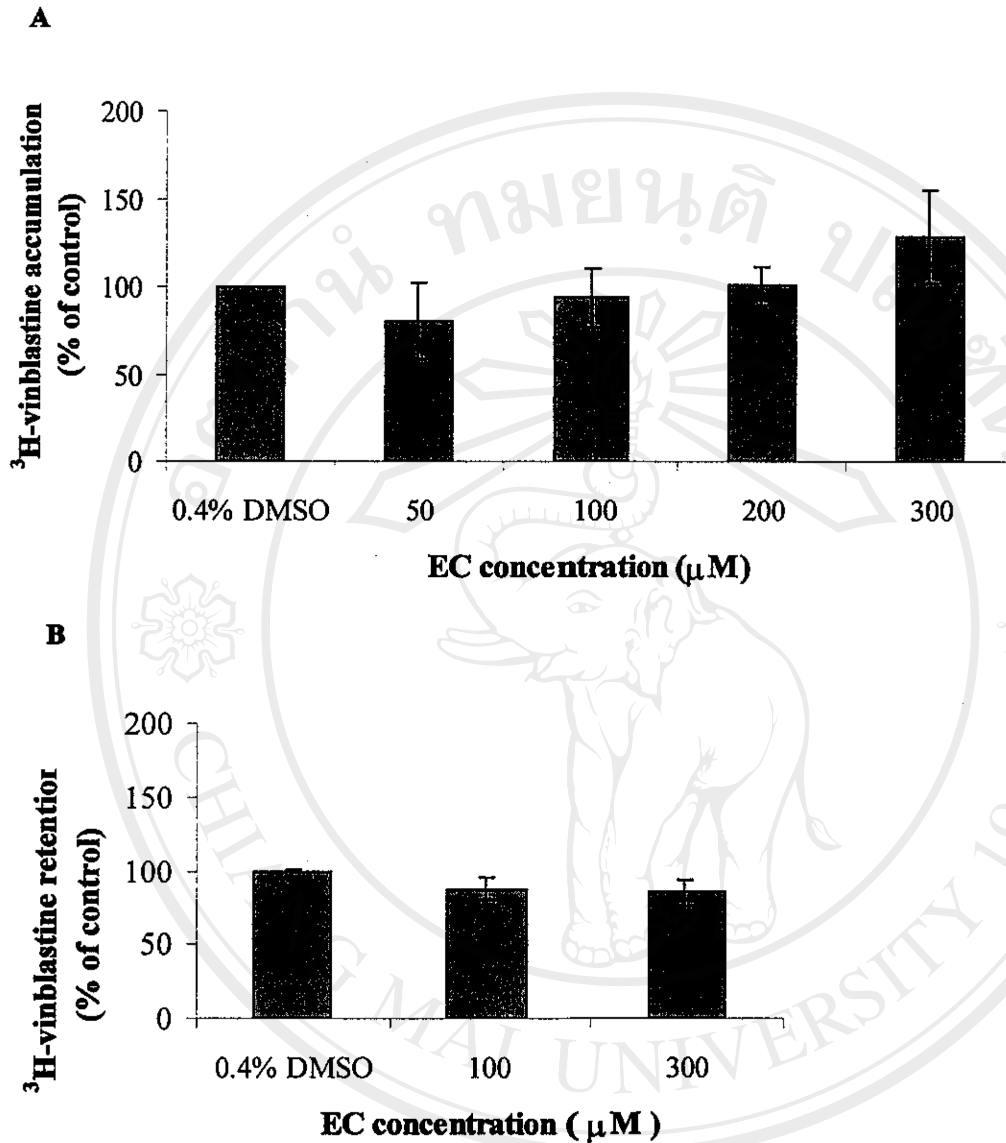

Figure 20. Effect of EC on ^{3}H-vinblastine accumulation (A) and efflux (B) in KB-V1 cell line. The amount of intracellular radioactivity was measured by the β counter. Each point represented the mean value of three times independent experiments performed in triplicate.

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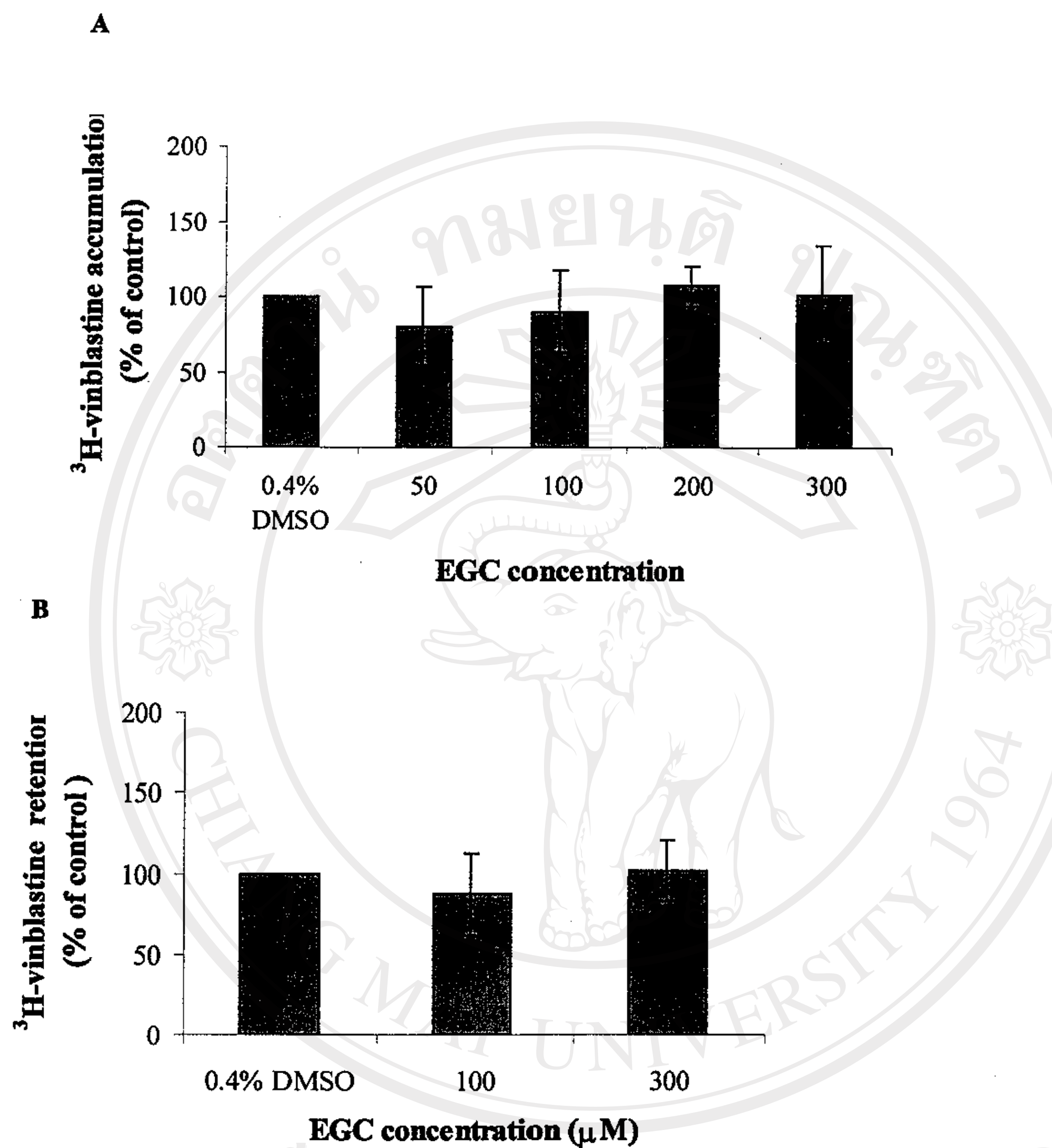

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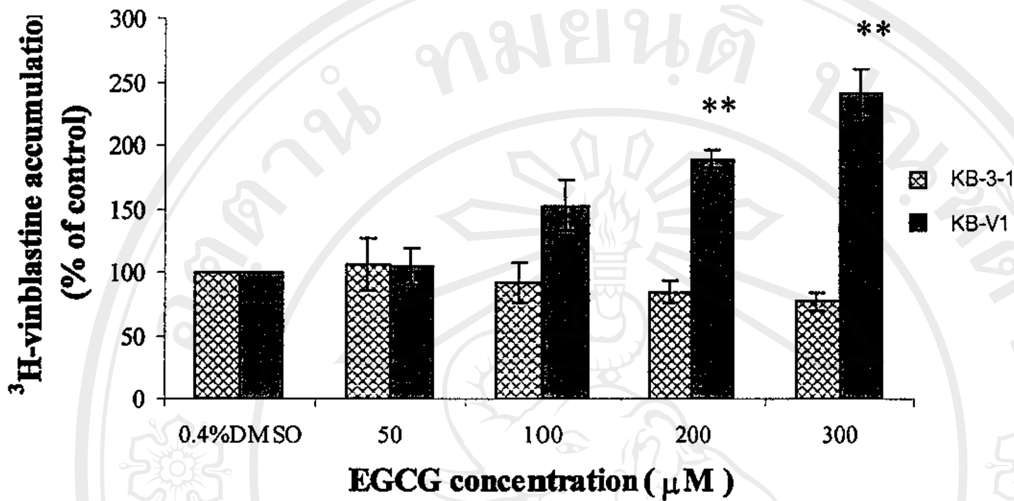
Figure 21. Effect of EGC on ^{3}H-vinblastine accumulation (A) and efflux (B) in KB-V1 cell line. The amount of intracellular radioactivity was measured by the β counter. Each point represented the mean value of three times independent experiments performed in duplicate.

A



B

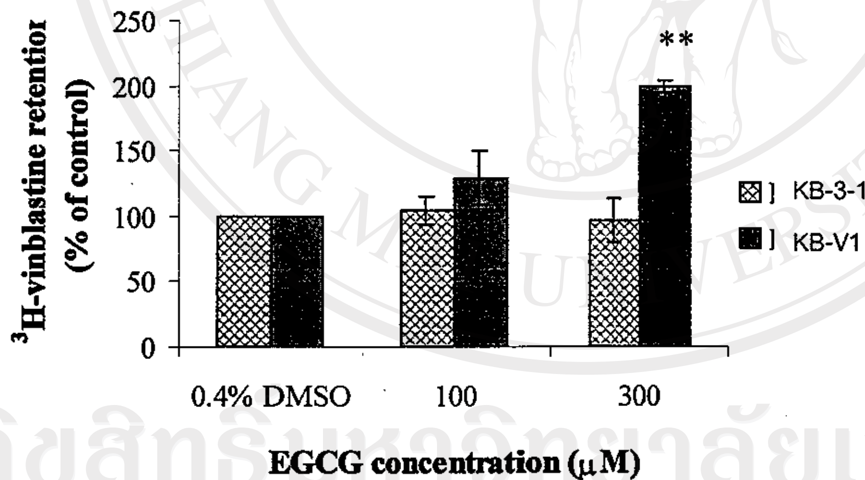


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Figure 22. Effect of EGCG on ^{3}H-vinblastine accumulation (A) and efflux (B) in KB-V1 and KB-3-1 cell lines. The amount of intracellular radioactivity was measured by the β counter. Each point represented the mean value of three times independent experiments performed in triplicate. Significant difference from untreated control was indicated by $p<0.01$ (**)

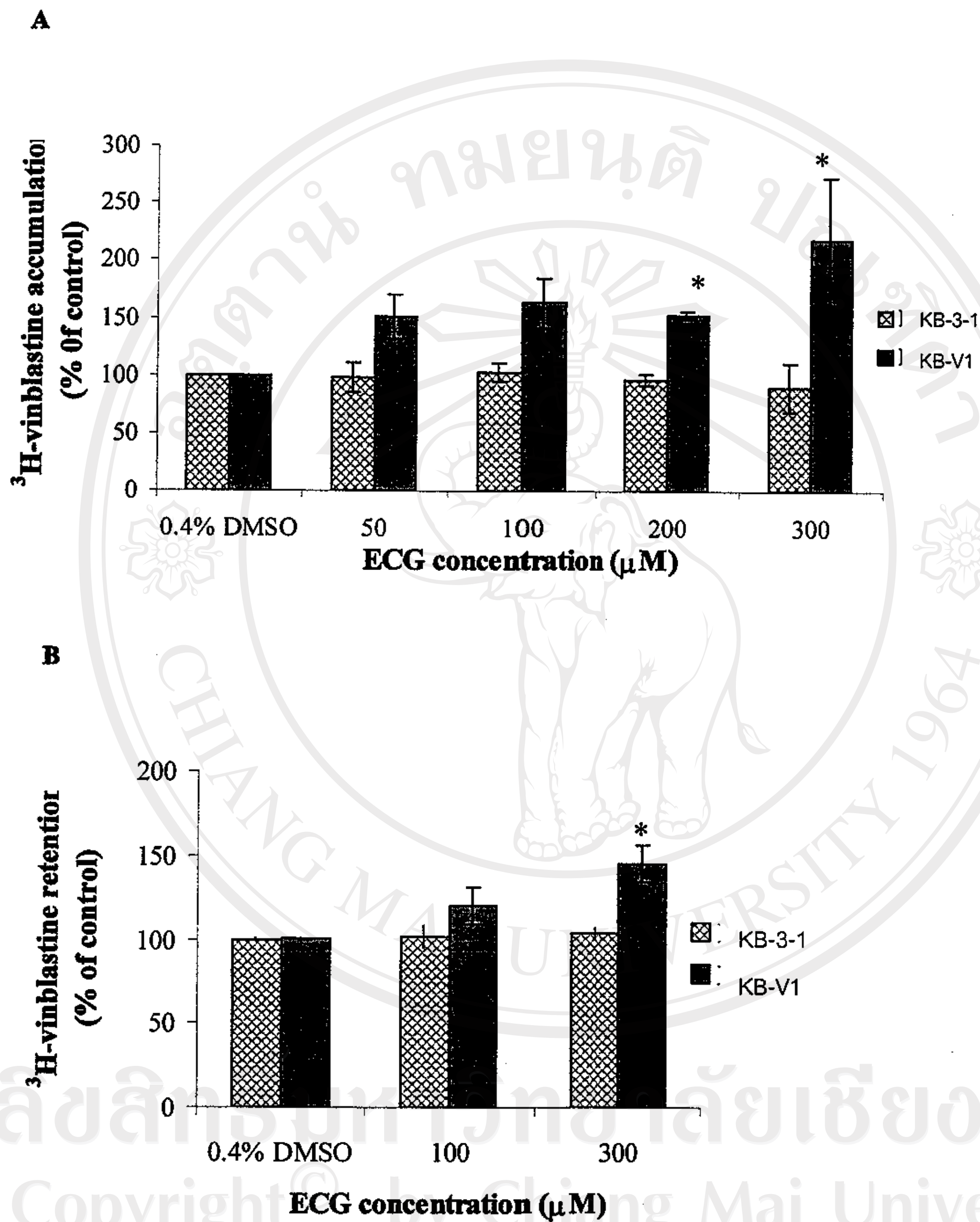

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Figure 23. Effect of ECG on ^{3}H-vinblastine accumulation (A) and efflux (B) in KB-V1 and KB-3-1 cell lines. The amount of intracellular radioactivity was measured by the β counter. Each point represented the mean value of three times independent experiments performed in triplicate. Significant difference from untreated control was indicated by $p<0.05$ (*)

Table 15. Effect of green tea flavonoids on ^{3}H-vinblastine accumulation in KB-V1 and KB-3-1 cells. The data showed in Figure 19-23 represented mean ± standard deviation of three independent experiments performed in triplicate.

Treatment	Concentration (μM)	^{3}H-vinblastine accumulation (% of control)	
		KB-V1	KB-3-1
catechin	0	100 ±0.0	-
	50	96 ±26	-
	100	101 ±15	-
	200	90 ±28	-
	300	110 ±20	-
Verapamil	30	408 ±46**	-
EC	0	100 ±0.0	-
	50	81 ±2.2	-
	100	94 ±17	-
	200	101 ±10	-
	300	129 ±26	-
Verapamil	30	447 ±84**	-
EGC	0	100 ±0.0	-
	50	80 ±26	-
	100	90 ±27	-
	200	107 ±13	-
	300	101 ±32	-
Verapamil	30	427 ±13**	-
EGCG	0	100 ±0.0	100 ±0.0
	50	105 ±15	106 ±21
	100	153 ±20	92 ±16
	200	190 ±7**	85 ±9
	300	242 ±19**	77 ±7
Verapamil	30	448 ±5**	119 ±8
ECG	0	100 ±0.0	100 ±0.0
	50	151 ±20	98 ±13
	100	164 ±22	102 ±8
	200	153 ±3*	96 ±5
	300	218 ±55*	99 ±21
Verapamil	30	480 ±134**	114 ±10

* Asterisks denoted values that were significantly different from the vehicle control ($P<0.05$).

** Asterisks denoted values that were significantly different from the vehicle control ($P<0.01$).

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Table 16. Effect of green tea flavonoids on ^{3}H-vinblastine efflux in KB-V1 and KB-3-1 cells. The amount of intracellular radioactivity was measured determined by the β counter. The data shown in Figure 19-23 represented mean ± standard deviation of three independent experiments performed in triplicate.

Treatment	Concentration (μM)	^{3}H-vinblastine retention (% of control)	
		KB-V1	KB-3-1
catechin	0	100 ± 0.0	-
	100	96 ± 18	-
	300	102 ± 22	-
Verapamil	30	416 ± 189**	-
EC	0	100 ± 0.0	-
	100	87 ± 8	-
	300	85 ± 9	-
Verapamil	30	244 ± 51**	-
EGC	0	100 ± 0.0	-
	100	87 ± 26	-
	300	102 ± 19	-
Verapamil	30	298 ± 37**	-
EGCG	0	100 ± 0.0	100 ± 0
	100	129 ± 20	105 ± 11
	300	200 ± 5**	97 ± 17
Verapamil	30	380 ± 57**	106 ± 15
ECG	0	100 ± 0.0	100 ± 0.0
	100	121 ± 11	102 ± 6
	300	145 ± 11*	104 ± 3
Verapamil	30	292 ± 61**	107 ± 7

* Asterisks denoted values that were significantly different from the vehicle control ($P < 0.05$).

** Asterisks denoted values that were significantly different from the vehicle control ($P < 0.01$).

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3.3 Effect of green tea flavonoids on Pgp expression (Pgp level) in KB-V1 cell line

3.3.1 Effect of ECG and EGCG on Pgp level in KB-V1 cell line at 2 h.

According to the results of ECG and EGCG that increased the intracellular Rh123 and ^{3}H-vinblastine in KB-V1 cells, the KB-V1 cells were incubated with ECG and EGCG for 2 h, the short time incubation which equaled to incubation periods in Pgp functional test. After plasma membrane preparation, 20µg/lane of the plasma membrane protein was separated on a 7.5% SDS-PAGE and Western blot analysis using Pgp specific antibody clone F4 was used to monitor the Pgp level. The results showed that both ECG and EGCG did not significantly affect the Pgp level when compared with 0.4%DMSO as a vehicle control in KB-V1 cells (Figure 24 and 25). The concentration of ECG and EGCG used in this experiment did not affect the cell viability and morphology when observed under phase contrast microscope because the incubation time was short and the cells were confluent. In KB-3-1 cells, the Pgp band was not detected by the western blot analysis, confirmed the drug sensitive phenomenon compared to the resistant KB-V1 which showed the high detectable level of Pgp.

3.3.2 Effect of green tea flavonoids on Pgp level inKB-V1 cells at 48 h.

To investigate the effect of green tea flavonoids on Pgp level, The KB-V1 cells were incubated with 0.4%DMSO or 50 and 100 µM green tea flavonoids for 48 h, then Western blot analysis was used to determine the level of Pgp. There was no significant difference in Pgp levels on catechin, EGC and EGCG treatment after 48 h incubation compared to 0.4%DMSO vehicle control (Figure 26-28). Treatment with 50 and 100 µM of EC significantly decreased the Pgp level in a dose dependent manner (Figure 29), while treatment with 50 and 100 µM of ECG significantly increased the Pgp level in a dose dependent manner (Figure 30). In KB-3-1 cells, the Pgp band was not detected by the western blot analysis compared to the resistant KB-V1 which showed the high detectable level of Pgp (Figure 24-30).

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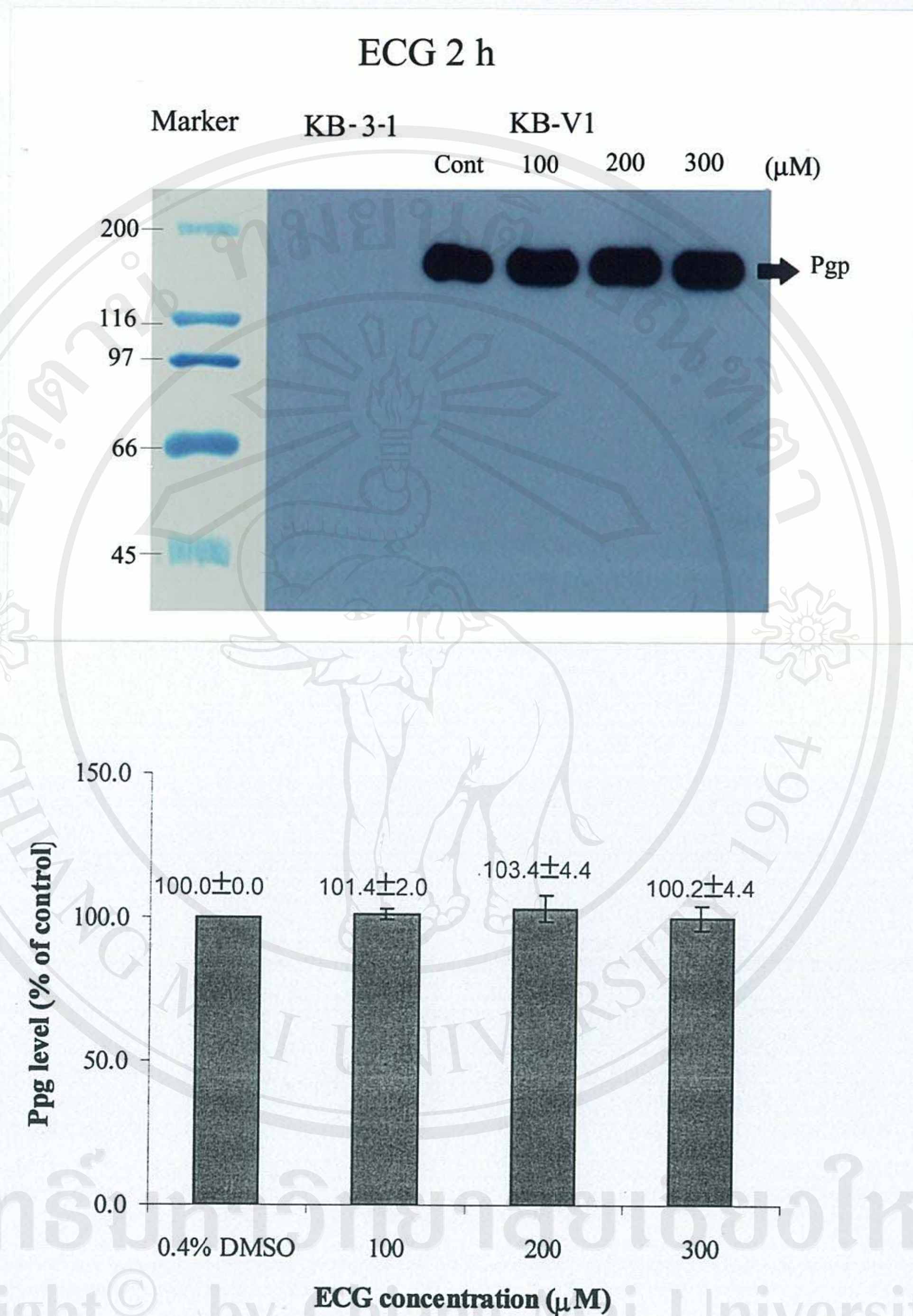

Figure 24. P-glycoprotein level in KB-V1 cells cultured in 100, 200 and 300 μM ECG for 2 h. Twenty microgram per lane of the plasma membrane proteins were separated on 7.5% SDS-PAGE and Western blot analysis, using Pgp specific antibody clone F4 was used to monitor the Pgp level (top) and then quantitated by densitometry (bottom). The data represented the mean values of three independent experiments performed in triplicate.

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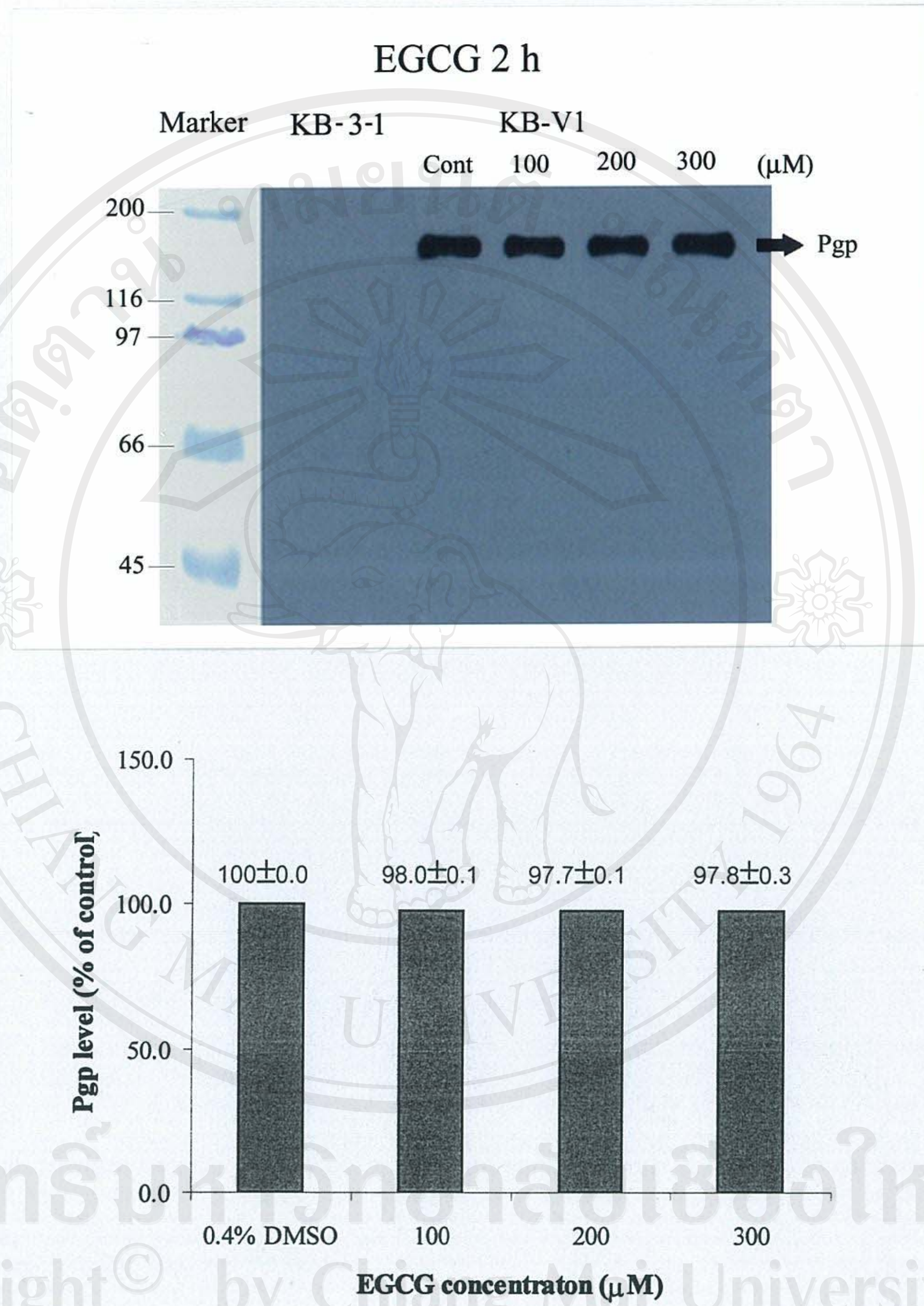

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Figure 25. P-glycoprotein level in KB-V1 cells cultured in 100, 200 and 300 μM EGCG for 2 h. Twenty microgram per lane of the plasma membrane proteins were separated on 7.5% SDS-PAGE and Western blot analysis using Pgp specific antibody clone F4 was used to monitor the Pgp level (top) and then quantitated by densitometry (bottom). The data represented the mean values of three independent experiments performed in triplicate.

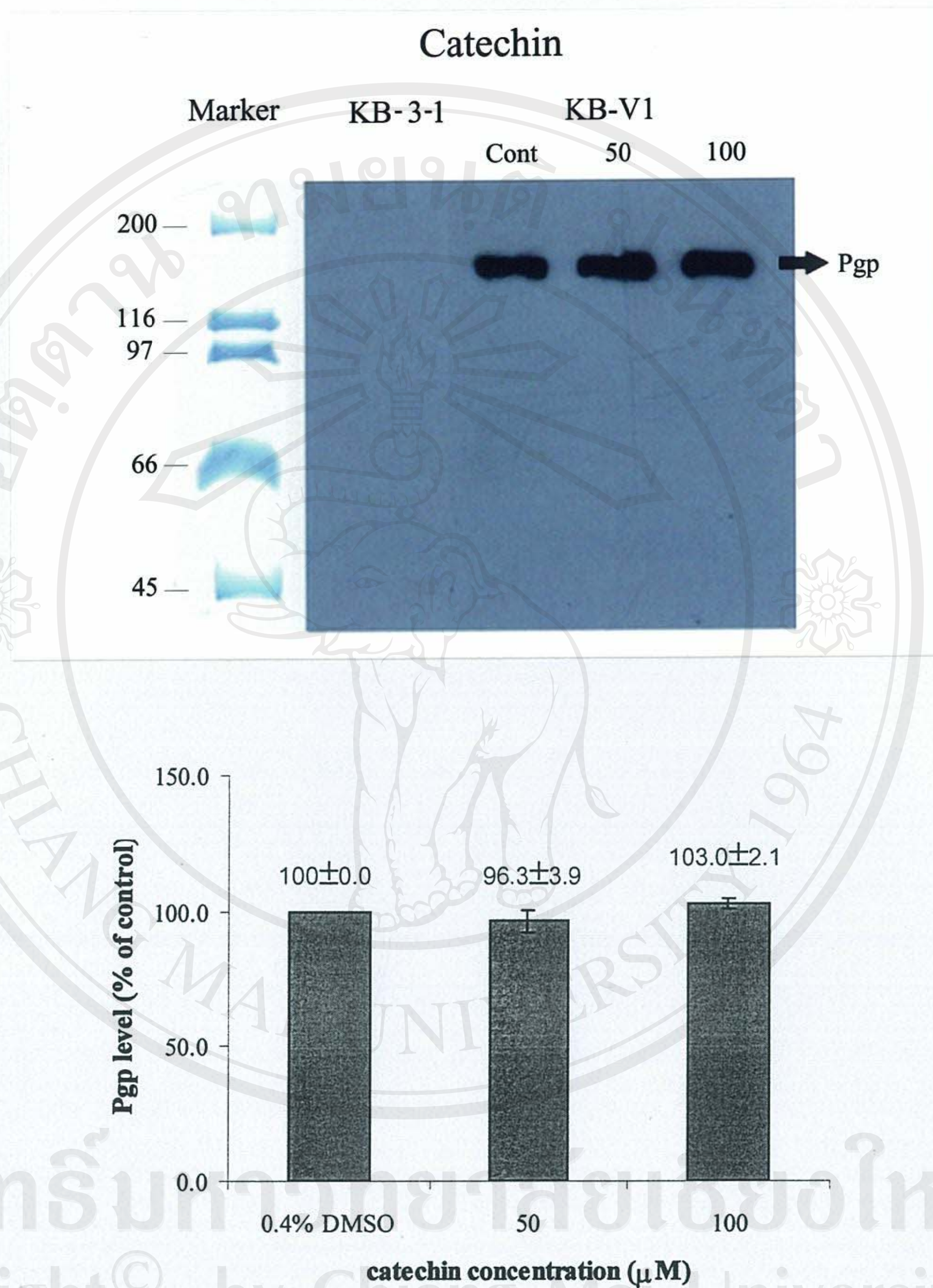

Figure 26. P-glycoprotein level in KB-V1 cells cultured in 50 and 100 μM catechin for 48 h. Twenty microgram per lane of the plasma membrane proteins were separated on 7.5% SDS-PAGE and Western blot analysis using Pgp specific antibody clone F4 was used to monitor the Pgp level (top) and then quantitated by densitometry (bottom). The data represented the mean values of three independent experiments performed in triplicate.

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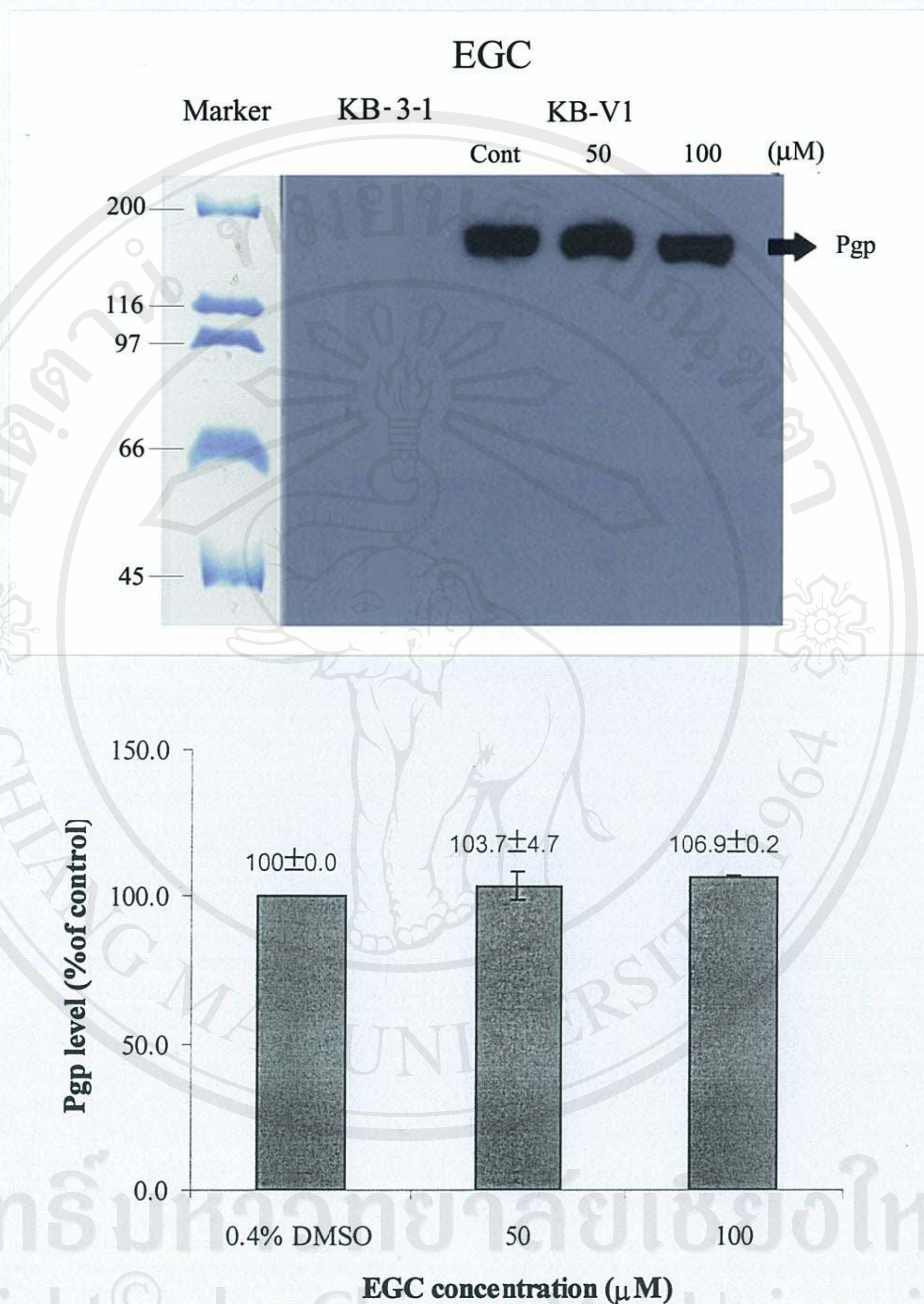

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Figure 27. P-glycoprotein level in KB-V1 cells cultured in 50 and 100 μM EGC for 48 h. Twenty microgram per lane of the plasma membrane proteins were separated on 7.5% SDS-PAGE and Western blot analysis using Pgp specific antibody clone F4 was used to monitor the Pgp level (top) and then quantitated by densitometry (bottom). The data represented the mean values of three independent experiments performed in triplicate.

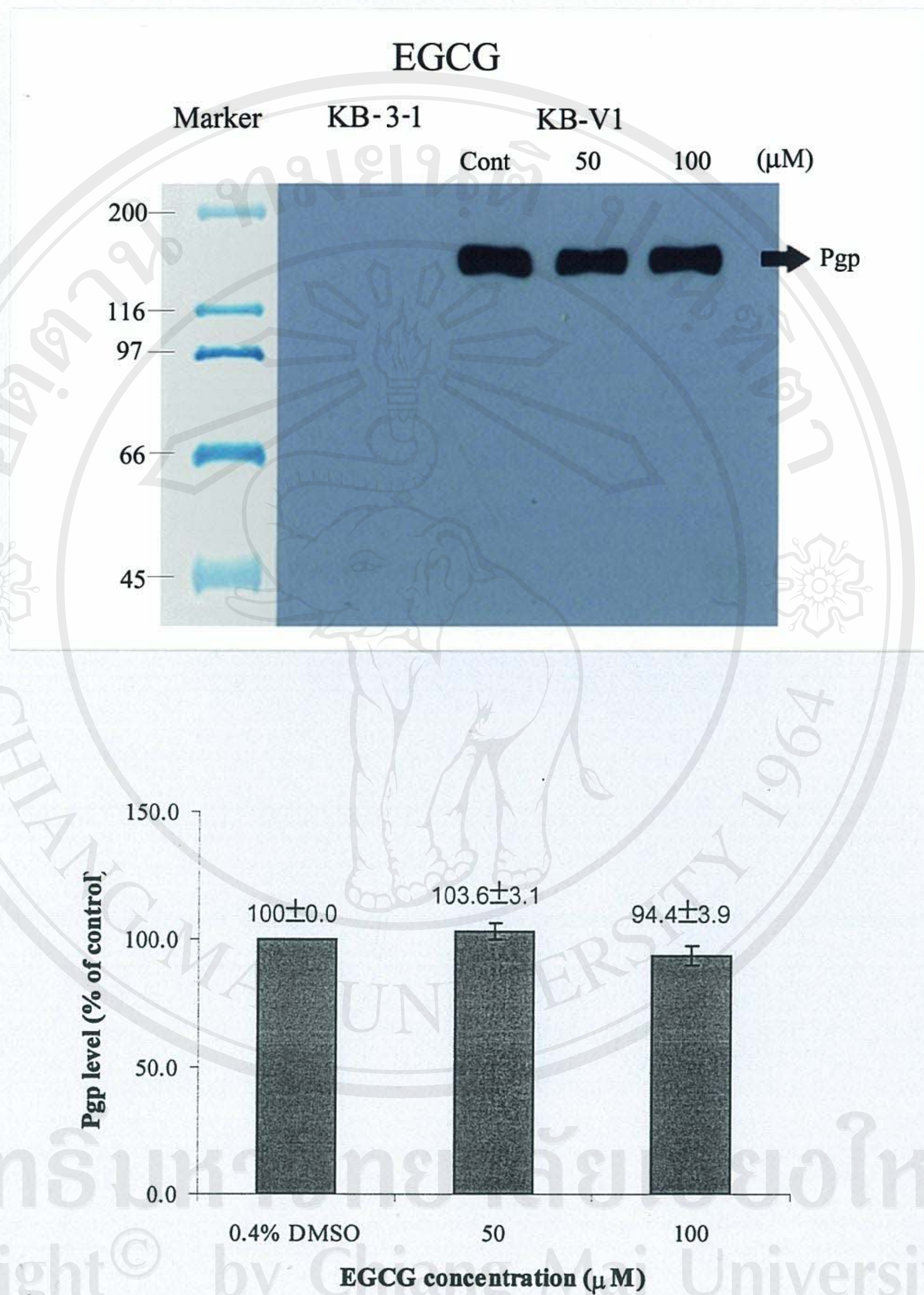

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Figure 28. P-glycoprotein level in KB-V1 cells cultured in 50 and 100 μM EGCG for 48 h. Twenty microgram per lane of the plasma membrane proteins were separated on 7.5% SDS-PAGE and Western blot analysis using Pgp specific antibody clone F4 was used to monitor the Pgp level (top) and then quantitated by densitometry (bottom). The data represented the mean values of three independent experiments performed in triplicate.

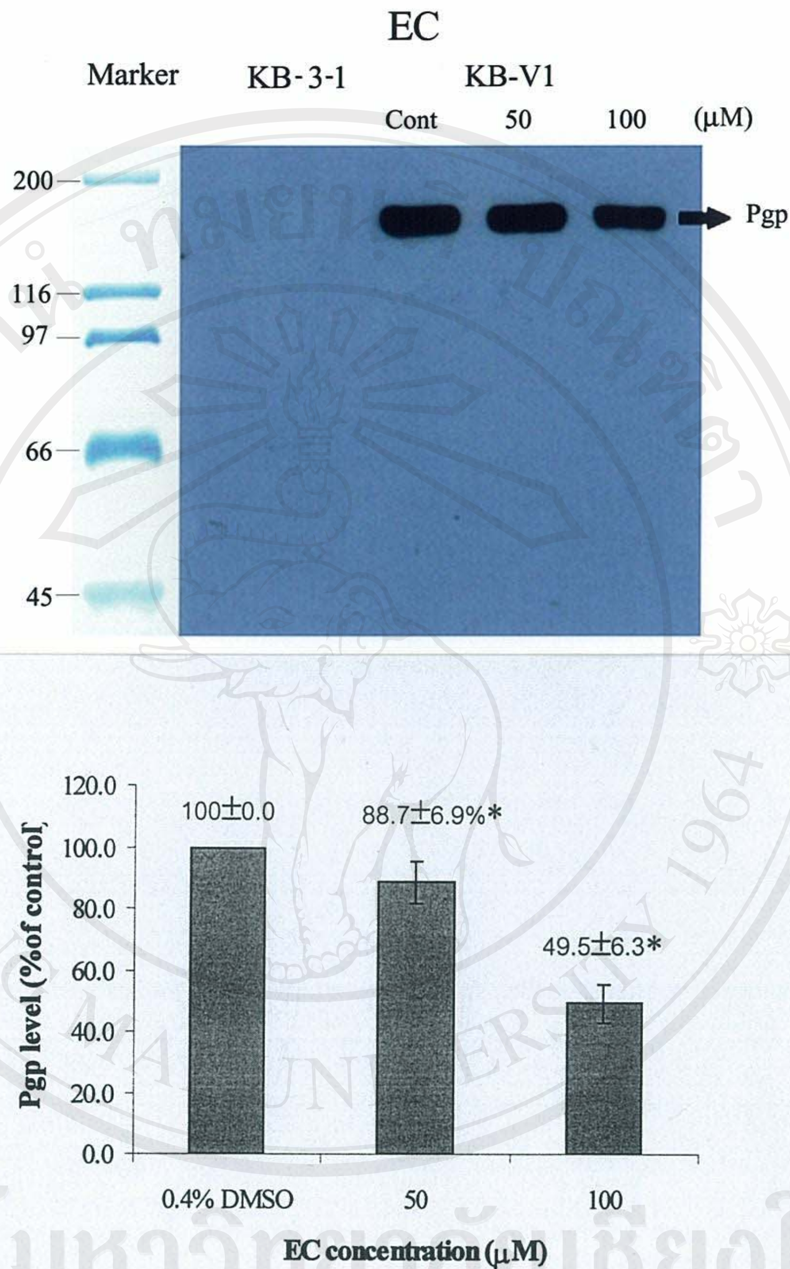

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Figure 29. P-glycoprotein level in KB-V1 cells cultured in 50 and 100 μM EC for 48 h. Twenty microgram per lane of the plasma membrane proteins were separated on 7.5% SDS-PAGE and Western blot analysis using Pgp specific antibody clone F4 was used to monitor the Pgp level (top) and then quantitated by densitometry (bottom). The data represented the mean values of three independent experiments performed in triplicate.

*Asterisks denoted values that were significantly different from the vehicle control ($P<0.01$)

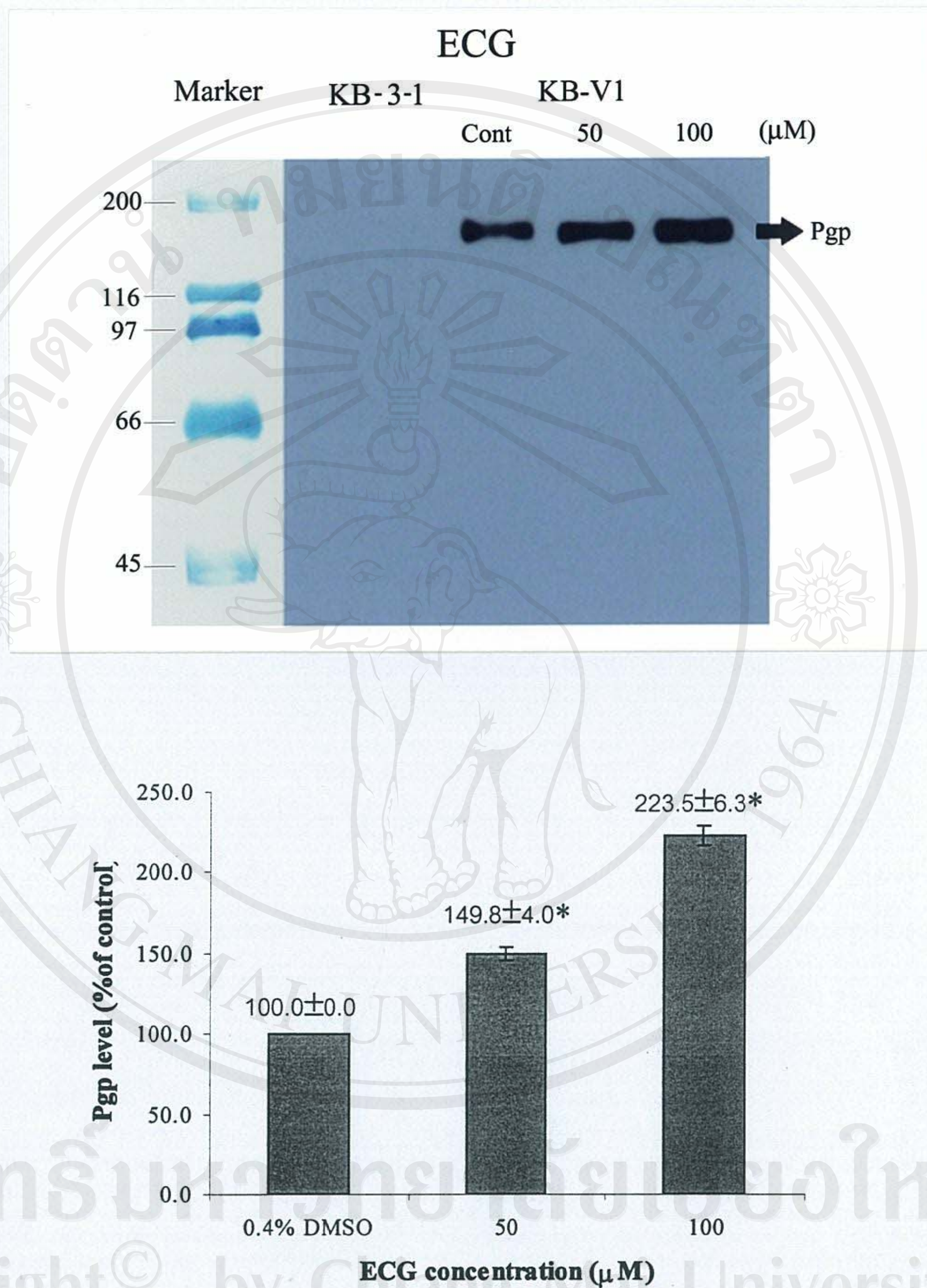

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Figure 30. P-glycoprotein level in KB-V1 cells cultured in 50 and 100 μM ECG for 48 h. Twenty microgram per lane of the plasma membrane proteins were separated on 7.5% SDS-PAGE and Western blot analysis using Pgp specific antibody clone F4 was used to monitor the Pgp level (top) and then quantitated by densitometry (bottom). The data represented the mean values of three independent experiments performed in triplicate.

*Asterisks denoted values that were significantly different from the vehicle control ($P<0.001$)

3.4 Effect of green tea flavonoids on cytotoxicity of chemotherapeutic drugs (MDR phenotype) in KB-V1 and KB-3-1 cell lines

Green tea flavonoids were co-incubated with the increasing concentrations of vinblastine for 48 h and then the number of viable cells were measured by MTT assay to determine their ability to sensitize the resistant cells, KB-V1 to vinblasine. The concentrations of green tea flavonoids used in this experiment were 50 and 100 μM which caused the cell death not more than 80% as shown in Section 3.1. Co-incubation of various concentrations of vinblastine with catechin, EC, ECG and EGC for 48 h did not affect vinblastine induced cell death in the resistant KB-V1 cells (Figure 31-34 and Table 17-20).

In contrast, co-incubation of the increasing concentrations of vinblastine with EGCG for 48 h was significantly increased the cell viability ($P<0.05$) in a dose dependent manner in KB-V1 cells (Figure 35 and Table 21). EGCG also caused a significant desensitizing effect ($P<0.05$) in a dose dependent manner in the sensitive KB-3-1 cells (Figure 36 and Table 22). Moreover, the co-inbubation of EGCG with the other chemotherapeutic drugs such as doxorubicin, colchicine and paclitaxel were tested. The results found that EGCG not only desensitized KB-V1 to vinblastine toxicity, but also significantly desensitized to doxorubicin, colchicine and paclitaxel in both the resistant KB-V1 cells and the sensitive KB-3-1 cells (Figure 37-42 and Table 23-28).

Because the concentration that affected the Pgp function were higher than 100 μM, the higher concentration of EGCG were incubated with the increasing concentration of vinblastine for 24 h. Desensitizing to vinblastine toxicity caused by EGCG could also be observed after 24 h incubation in a dose dependent manner in KB-V1 cells (Figure 43 and Table 29).

However, pre-incubation with EGCG alone for 48h and then treated with the increasing concentrations of vinblastine for another 48h caused no significant effect on vinblastine induced cell death as shown in Figure 44 and Table 30.

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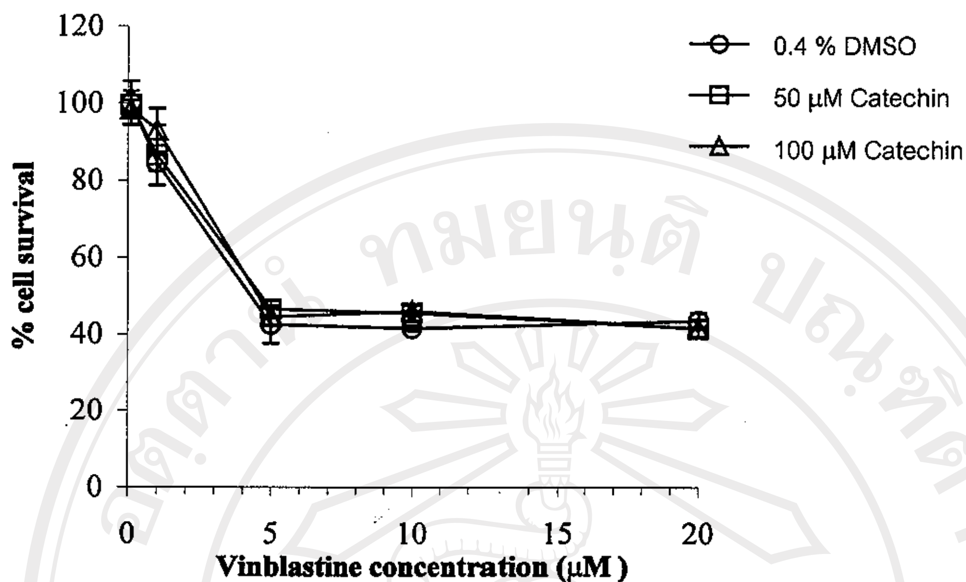

Figure 31. Effect of catechin on vinblastine cytotoxicity in KB-V1 cells. KB-V1 cells were co-incubated with the increasing concentrations of vinblastine and 0.4%DMSO as vehicle control, 50 and 100 μM catechin. After 48 h incubation, the number of viable cells were determined by MTT assay. Each point represents the mean value of three independent experiments performed in triplicate.

Table 17. Effect of catechin on vinblastine cytotoxicity in KB-V1 cells. The data showed in Figure 31 represented mean ± standard deviation of three independent experiments performed in triplicate.

Vinblastine concentration (μM)	% cell survival		
	0.4%DMSO	50 μM catechin	100 μM catechin
0	100 ± 0	100 ± 0	100 ± 0
0.1	100 ± 6	100 ± 4	99 ± 2
1	85 ± 2	87 ± 8	93 ± 6
5	43 ± 5	47 ± 2	45 ± 2
10	42 ± 1	46 ± 1	46 ± 1
20	44 ± 2	42 ± 2	42 ± 2

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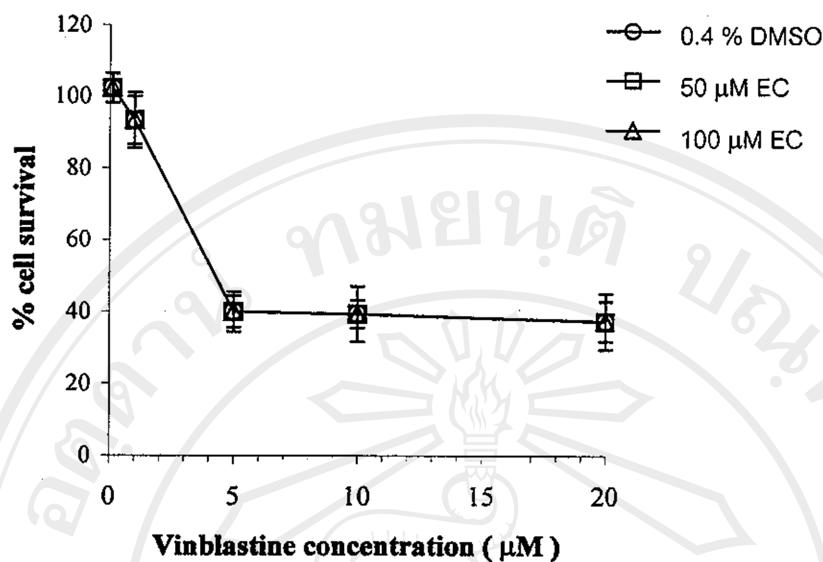

Figure 32. Effect of EC on vinblastine cytotoxicity in KB-V1 cells. KB-V1 cells were co-incubated with the increasing concentrations of vinblastine and 0.4%DMSO as vehicle control, 50 and 100 μM EC. After 48h incubation, the number of viable cells were determined by MTT assay. Each point represents the mean value of three independent experiments performed in triplicate.

Table 18. Effect of EC on vinblastine cytotoxicity in KB-V1 cells. The data showed in Figure 32 represented mean ± standard deviation of three independent experiments performed in triplicate.

Vinblastine concentration (μM)	% cell survival		
	0.4%DMSO	50 μM EC	100 μM EC
0	100 ± 0	100 ± 0	100 ± 0
0.1	102 ± 4	102 ± 4	103 ± 4
1	93 ± 8	94 ± 11	93 ± 7
5	40 ± 6	41 ± 7	43 ± 4
10	39 ± 4	40 ± 5	37 ± 8
20	37 ± 6	37 ± 6	36 ± 8

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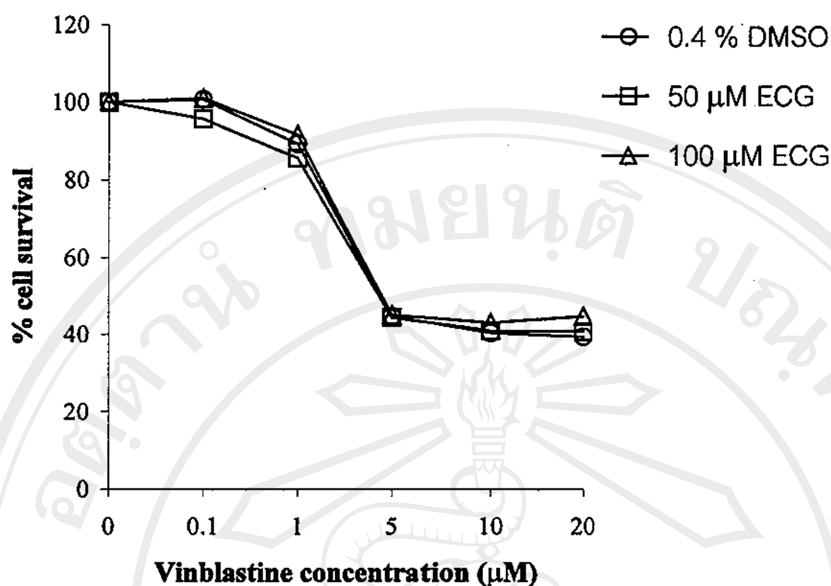

Figure 33. Effect of ECG on vinblastine cytotoxicity in KB-V1 cells. KB-V1 cells were co-incubated with the increasing concentrations of vinblastine and 0.4%DMSO as vehicle control, 50 and 100 μM ECG. After 48 h incubation, the number of viable cells were determined by MTT assay. Each point represented the mean value of three independent experiments performed in triplicate.

Table 19. Effect of ECG on vinblastine cytotoxicity in KB-V1 cells. The data showed in Figure 33 represented mean ± standard deviation of three independent experiments performed in triplicate.

Vinblastine concentration (μM)	% cell survival		
	0.4%DMSO	50 μM ECG	100 μM ECG
0	100 ± 0	100 ± 0	100 ± 0
0.1	101 ± 1	96 ± 5	101 ± 2
1	89 ± 3	86 ± 3	92 ± 5
5	45 ± 3	44 ± 2	45 ± 2
10	40 ± 4	41 ± 2	43 ± 1
20	39 ± 3	41 ± 2	45 ± 4

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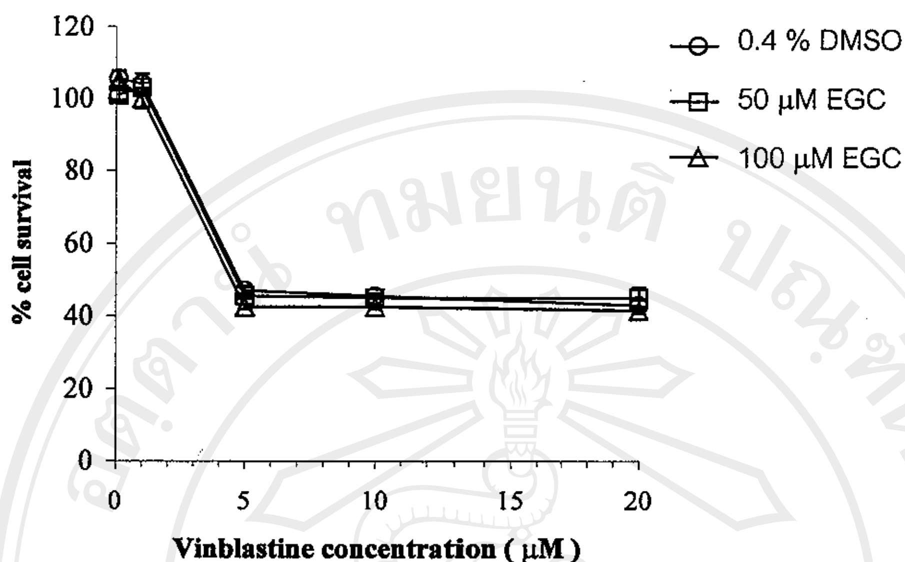

Figure 34. Effect of EGC on vinblastine cytotoxicity in KB-V1 cells. KB-V1 cells were co-incubated with the increasing concentrations of vinblastine and 0.4%DMSO as vehicle control, 50 and 100 μM EGC. After 48 h incubation, the number of viable cells were determined by MTT assay. Each point represented the mean value of three independent experiments performed in triplicate.

Table 20. Effect of EGC on vinblastine cytotoxicity in KB-V1 cells. The data showed in Figure 34 represented mean ± standard deviation of three independent experiments performed in triplicate.

Vinblastine concentration (μM)	% cell survival		
	0.4%DMSO	50 μM EGC	100 μM EGC
0	100 ± 0	100 ± 0	100 ± 0
0.1	106 ± 2	101 ± 1	105 ± 3
1	104 ± 3	103 ± 1	100 ± 3
5	47 ± 1	46 ± 1	43 ± 1
10	46 ± 1	45 ± 0	43 ± 1
20	43 ± 1	45 ± 3	42 ± 1

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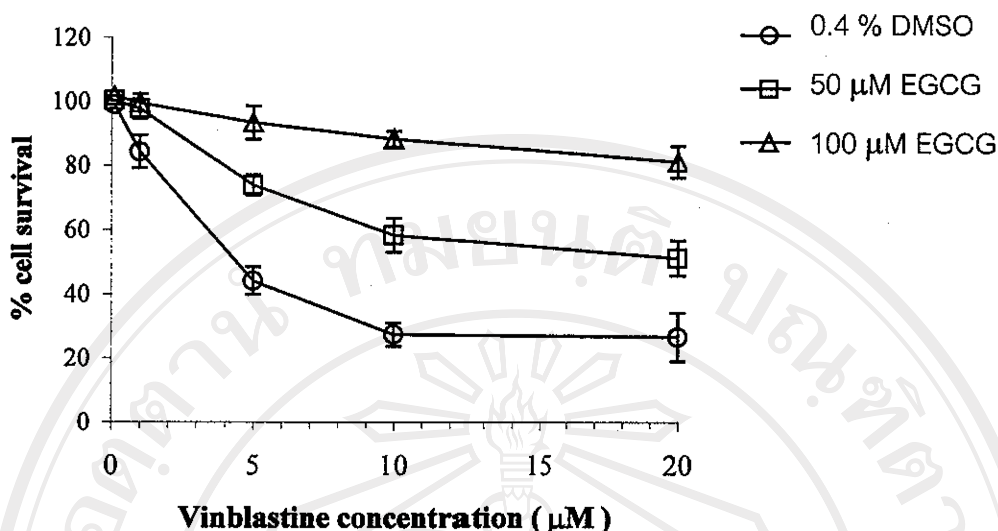

Figure 35. Effect of EGCG on vinblastine cytotoxicity in KB-V1 cells. KB-V1 cells were co-incubated with the increasing concentrations of vinblastine and 0.4%DMSO as vehicle control, 50 and 100 μM EGCG. After 48 h incubation, the number of viable cells were determined by MTT assay. Each point represented the mean value of three independent experiments performed in triplicate.

Table 21. Effect of EGCG on vinblastine cytotoxicity in KB-V1 cells. The data showed in Figure 35 represented mean ± standard deviation of three independent experiments performed in triplicate.

Vinblastine concentration (μM)	% cell survival		
	0.4%DMSO	50μM EGCG	100μM EGCG
0	100 ± 0	100 ± 0	100 ± 0
0.1	99 ± 5	100 ± 3	102 ± 3
1	84 ± 4	98 ± 3*	99 ± 5*
5	44 ± 4	74 ± 5*	93 ± 2*
10	37 ± 8	58 ± 5*	88 ± 5*
20	27 ± 8	51 ± 10*	81 ± 4*

*Asterisks denoted values that were significantly different from the vehicle control (0.4% DMSO) at each vinblastine concentration ($P<0.05$).

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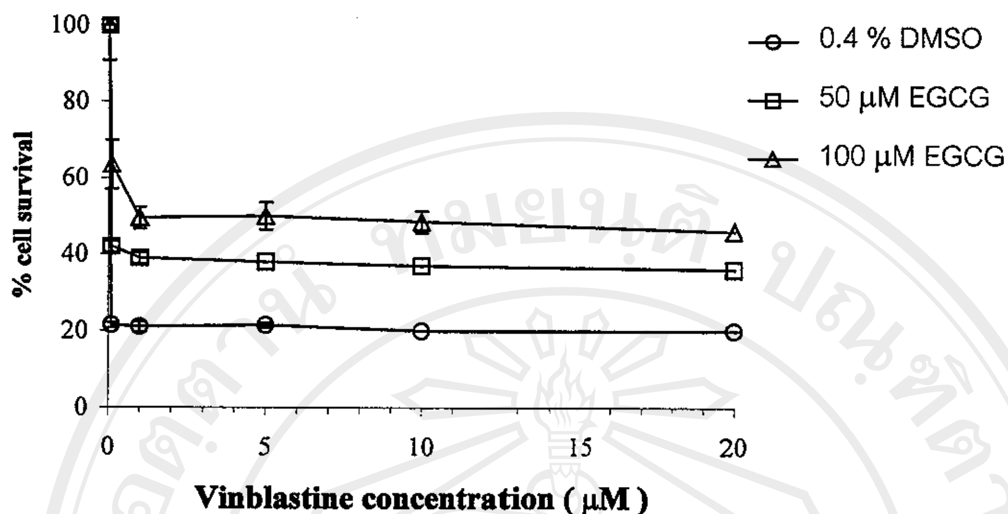

Figure 36. Effect of EGCG on vinblastine cytotoxicity in KB-3-1 cells. KB-3-1 cells were co-incubated with the increasing concentrations of vinblastine and 0.4%DMSO as vehicle control, 50 and 100 μM EGCG. After 48 h incubation, the number of viable cells were determined by MTT assay. Each point represented the mean value of three independent experiments performed in triplicate.

Table 22. Effect of EGCG on vinblastine cytotoxicity in KB-3-1 cells. The data showed in Figure 36 represented mean ± standard deviation of three independent experiments performed in triplicate.

Vinblastine concentration (μM)	% cell survival		
	0.4%DMSO	50 μM EGCG	100 μM EGCG
0	100 ± 0	100 ± 0	100 ± 0
0.1	22 ± 1	42 ± 1*	64 ± 9*
1	21 ± 1	39 ± 1*	50 ± 6*
5	22 ± 1	38 ± 1*	50 ± 3*
10	20 ± 0	37 ± 0*	49 ± 4*
20	20 ± 0	36 ± 0*	46 ± 3*

*Asterisks denoted values that were significantly different from the vehicle control (0.4% DMSO) at each vinblastine concentration ($P<0.05$).

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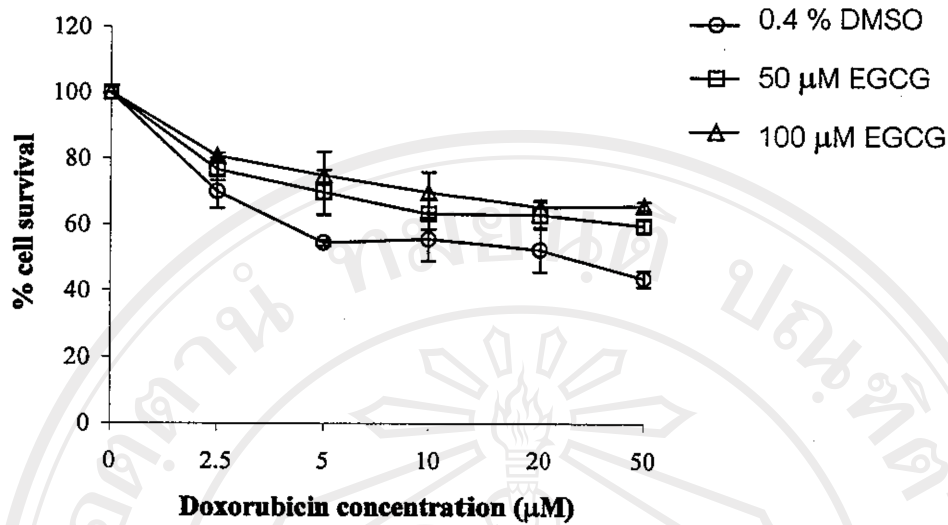

Figure 37. Effect of EGCG on doxorubicin cytotoxicity in KB-V1 cells. KB-V1 cells were co-incubated with the increasing concentrations of doxorubicin and 0.4%DMSO as vehicle control, 50 and 100 μM EGCG. After 48 h incubation, the number of viable cells were determined by MTT assay. Each point represented the mean value of three independent experiments performed in triplicate.

Table 23. Effect of EGCG on doxorubicin cytotoxicity in KB-V1 cells. The data showed in Figure 37 represented mean ± standard deviation of three independent experiments performed in triplicate.

doxorubicin concentration (μM)	% cell survival		
	0.4%DMSO	50 μM EGCG	100 μM EGCG
0	100 ± 0	100 ± 0	100 ± 0
2.5	70 ± 5	77 ± 3	81 ± 1*
5	54 ± 1	70 ± 7	75 ± 7*
10	56 ±7	63 ± 5*	70 ± 6*
20	52 ± 7	63 ± 4*	65 ± 2*
50	44 ± 2	60 ± 2*	66 ± 1*

*Asterisks denoted values that were significantly different from the vehicle control (0.4% DMSO) at each doxorubicin concentration ($P<0.05$).

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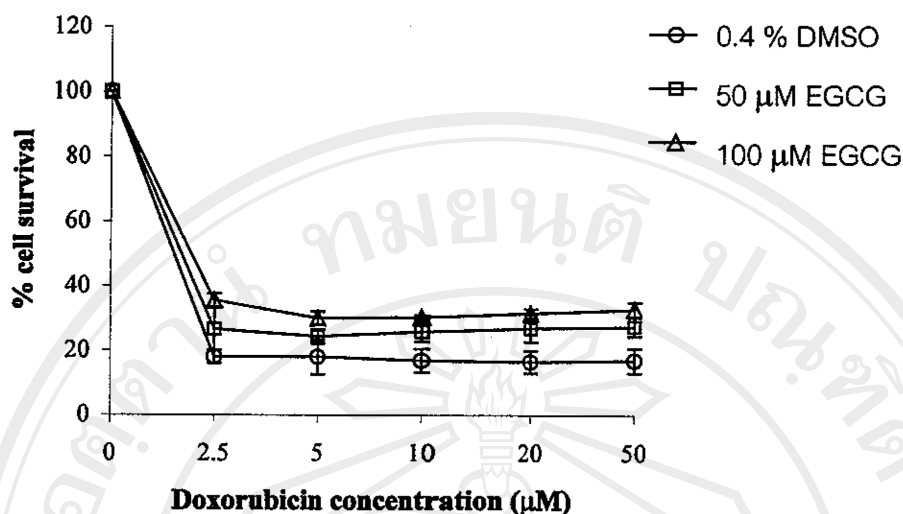

Figure 38. Effect of EGCG on doxorubicin cytotoxicity in KB-3-1 cells. KB-3-1 cells were co-incubated with the increasing concentrations of doxorubicin and 0.4%DMSO as vehicle control, 50 and 100 μM EGCG. After 48 h incubation, the number of viable cells were determined by MTT assay. Each point represented the mean value of three independent experiments performed in triplicate.

Table 24. Effect of EGCG on doxorubicin cytotoxicity in KB-3-1 cells. The data showed in Figure 38 represented mean ± standard deviation of three independent experiments performed in triplicate.

doxorubicin concentration (μM)	% cell survival		
	0.4%DMSO	50 μM EGCG	100 μM EGCG
0	100 ± 0	100 ± 0	100 ± 0
2.5	18 ± 2	27 ± 7	36 ± 2*
5	18 ± 2	24 ± 2	30 ± 2*
10	17 ± 4	26 ± 3*	30 ± 1*
20	17 ± 3	27 ± 4*	32 ± 1*
50	17 ± 4	27 ± 3*	33 ± 1*

*Asterisks denoted values that were significantly different from the vehicle control (0.4% DMSO) at each doxorubicin concentration ($P<0.05$).

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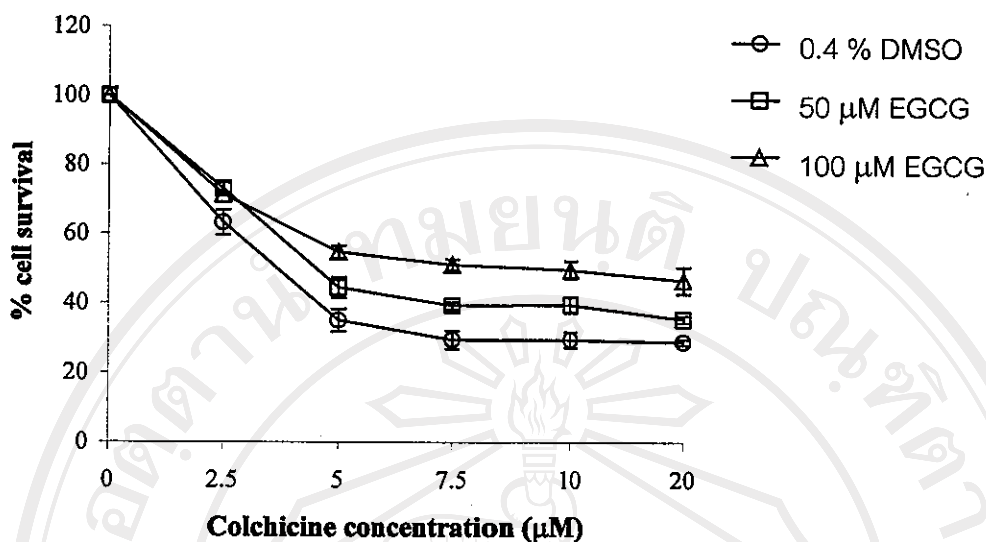

Figure 39. Effect of EGCG on colchicine cytotoxicity in KB-V1 cells. KB-V1 cells were co-incubated with the increasing concentrations of colcicine and 0.4%DMSO as vehicle control, 50 and 100 μM EGCG. After 48 h incubation, the number of viable cells were determined by MTT assay. Each point represented the mean value of three independent experiments performed in triplicate.

Table 25. Effect of EGCG on colchicine cytotoxicity in KB-V1 cells. The data showed in Figure 39 represented mean ± standard deviation of three independent experiments performed in triplicate.

colchicine concentration (μM)	% cell survival		
	0.4%DMSO	50 μM EGCG	100 μM EGCG
0	100 ± 0	100 ± 0	100 ± 0
2.5	63 ± 4	73 ± 2*	71 ± 0*
5	35 ± 3	44 ± 3*	55 ± 2*
7.5	29 ± 3	39 ± 1*	51 ± 2*
10	29 ± 2	39 ± 2*	50 ± 3*
20	29 ± 1	35 ± 1*	46 ± 4*

*Asterisks denoted values that were significantly different from the vehicle control (0.4% DMSO) at each colchicine concentration ($P<0.05$).

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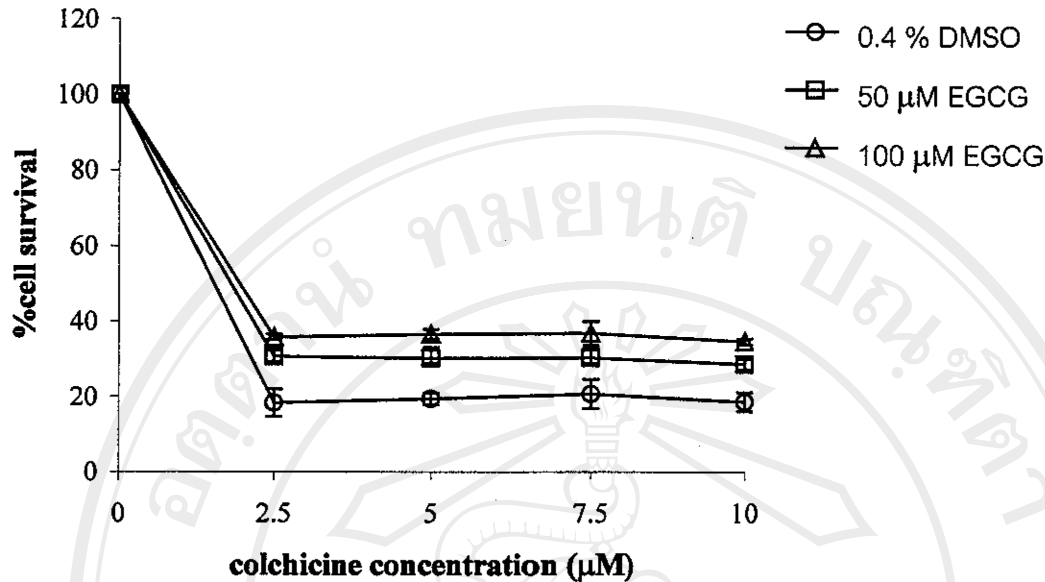

Figure 40. Effect of EGCG on colchicine cytotoxicity in KB-3-1 cells. KB-3-1 cells were co-incubated with the increasing concentrations of colcicine and 0.4%DMSO as vehicle control, 50 and 100 μM EGCG. After 48 h incubation, the number of viable cells were determined by MTT assay. Each point represented the mean value of three independent experiments performed in triplicate.

Table 26. Effect of EGCG on colchicine cytotoxicity in KB-3-1 cells. The data showed in Figure 40 represented mean ± standard deviation of three independent experiments performed in triplicate.

colchicine concentration (μM)	% cell survival		
	0.4%DMSO	50 μM EGCG	100 μM EGCG
0	100 ± 0	100 ± 0	100 ± 0
2.5	18 ± 4	31 ± 2*	36 ± 1*
5	19 ± 2	30 ± 3*	37 ± 1*
10	21 ± 4	30 ± 3*	37 ± 1*
20	19 ± 3	29 ± 1*	35 ± 1*

*Asterisks denoted values that were significantly different from the vehicle control (0.4% DMSO) at each colchicine concentration ($P<0.05$).

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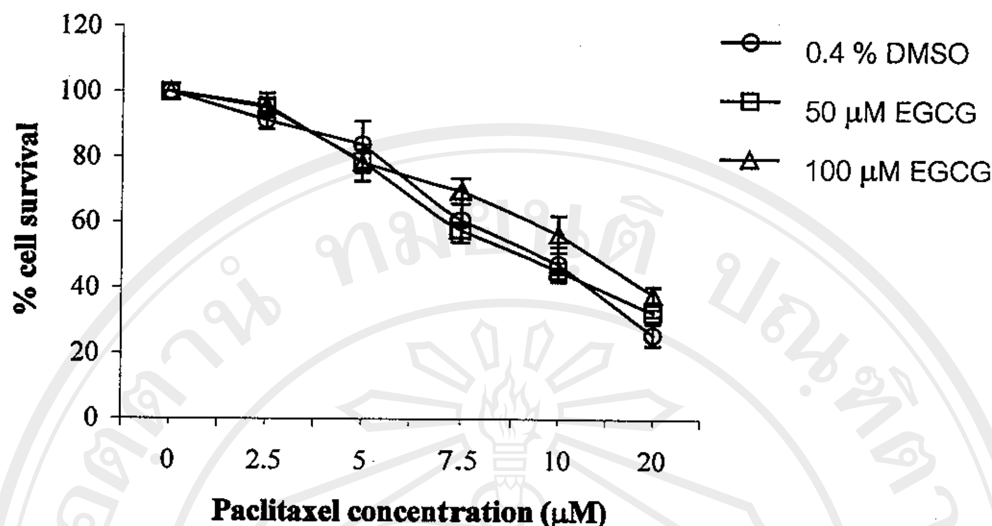

Figure 41. Effect of EGCG on paclitaxel cytotoxicity in KB-V1 cells. KB-V1 cells were co-incubated with the increasing concentrations of paclitaxel and 0.4%DMSO as vehicle control, 50 and 100 μM EGCG. After 48 h incubation, the number of viable cells were determined by MTT assay. Each point represented the mean value of three independent experiments performed in triplicate.

Table 27. Effect of EGCG on paclitaxel cytotoxicity in KB-V1 cells. The data showed in Figure 41 represented mean ± standard deviation of three independent experiments performed in triplicate.

paclitaxel concentration (μM)	% cell survival		
	0.4%DMSO	50 μM EGCG	100 μM EGCG
0	100 ± 0	100 ± 0	100 ± 0
2.5	91 ± 3	95 ± 3	96 ± 4
5	84 ± 7	79 ± 3	78 ± 6
7.5	61 ± 7	57 ± 2	70 ± 4
10	47 ± 5	45 ± 1	56 ± 6
20	26 ± 3	32 ± 1*	38 ± 2*

*Asterisks denoted values that were significantly different from the vehicle control (0.4% DMSO) at each paclitaxel concentration ($P<0.05$).

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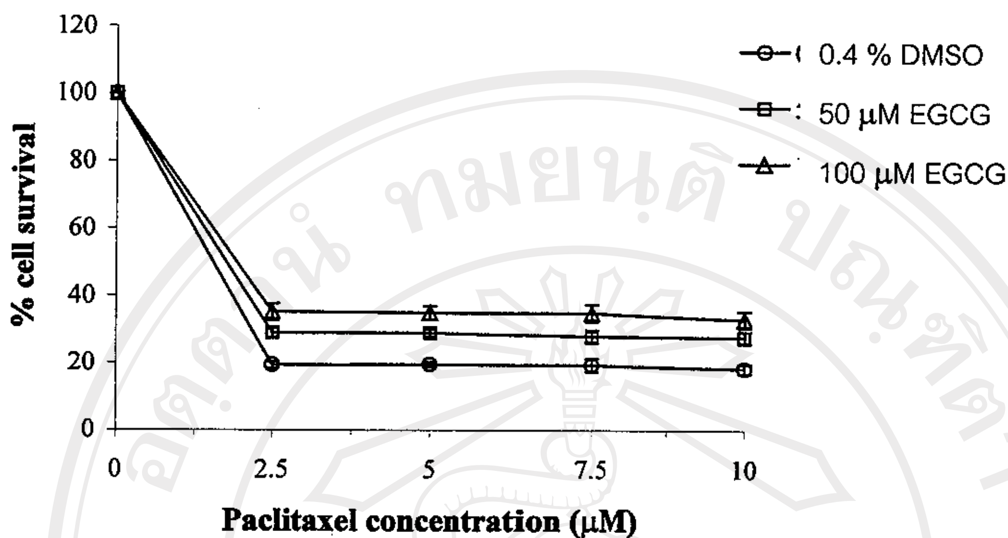

Figure 42. Effect of EGCG on paclitaxel cytotoxicity in KB-3-1 cells. KB-3-1cells were co-incubated with the increasing concentrations of paclitaxel and 0.4%DMSO as vehicle control, 50 and 100 µM EGCG. After 48 h incubation, the number of viable cells were determined by MTT assay. Each point represented the mean value of three independent experiments performed in triplicate.

Table 28. Effect of EGCG on paclitaxel cytotoxicity in KB-3-1 cells. The data showed in Figure 42 represented mean ± standard deviation of three independent experiments performed in triplicate.

paclitaxel concentration (µM)	% cell survival		
	0.4%DMSO	50 µM EGCG	100 µM EGCG
0	100 ± 0	100 ± 0	100 ± 0
2.5	19 ± 1	29 ± 1*	35 ± 2*
5	20 ± 1	29 ± 1*	35 ± 2*
7.5	19 ± 2	28 ± 2*	35 ± 3*
10	18 ± 1	28 ± 2*	33 ± 2*

*Asterisks denoted values that were significantly different from the vehicle control (0.4% DMSO) at each paclitaxel concentration ($P<0.05$).

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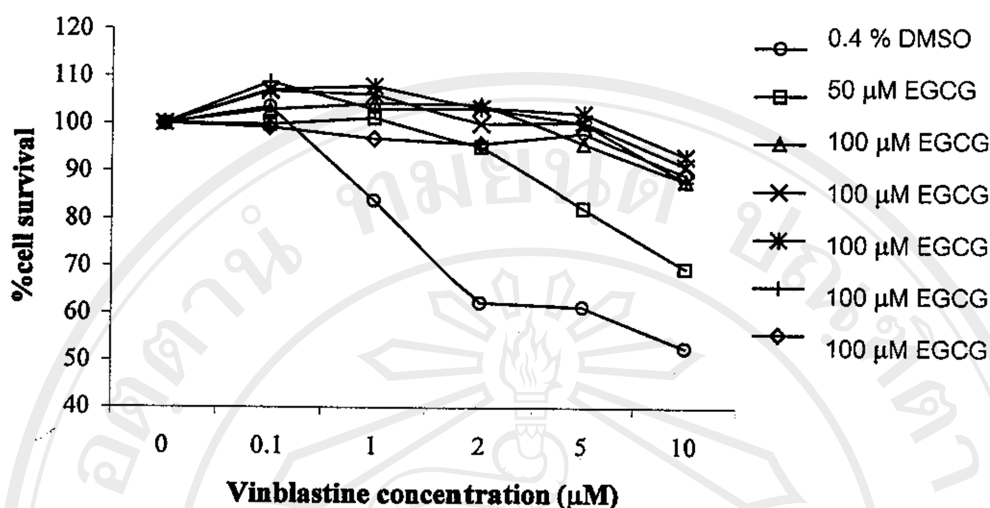

Figure 43. Effect of EGCG on vinblastine cytotoxicity in KB-V1 cells for 24 h incubation. KB-V1 cells were co-incubated with the increasing concentrations of vinblastine and 0.4%DMSO as vehicle control, 50 and 100 μM EGCG. After 24h incubation, the number of viable cells were determined by MTT assay. Each point represented the mean value of three independent experiments performed in triplicate.

Table 29. Effect of EGCG on vinblastine cytotoxicity in KB-V1 cells for 24 h incubation. The data showed in Figure 43 represented mean ± standard deviation of three independent experiments performed in triplicate.

Vinblastine concentration (μM)	% cell survival						
	0.4%DMSO	50	100	150	200	250	300
0	100 ± 0	100 ± 0	100 ± 0	100 ± 0	100 ± 0	100 ± 0	100 ± 0
0.1	104 ± 2	100 ± 2	103 ± 9	107 ± 2	107 ± 0	109 ± 2	99 ± 9
1	84 ± 4*	101 ± 5*	104 ± 3*	106 ± 3*	108 ± 2*	103 ± 4*	97 ± 6*
5	62 ± 6*	95 ± 3*	104 ± 3*	100 ± 1*	104 ± 3*	103 ± 3*	96 ± 4*
10	61 ± 8*	82 ± 3*	96 ± 4*	100 ± 4*	102 ± 5*	100 ± 3*	98 ± 5*
20	53 ± 8*	70 ± 2*	88 ± 11*	91 ± 4*	93 ± 4*	88 ± 1*	89 ± 2*

*Asterisks denoted values that were significantly different from the vehicle control (0.4% DMSO) at each vinblastine concentration ($P<0.05$).

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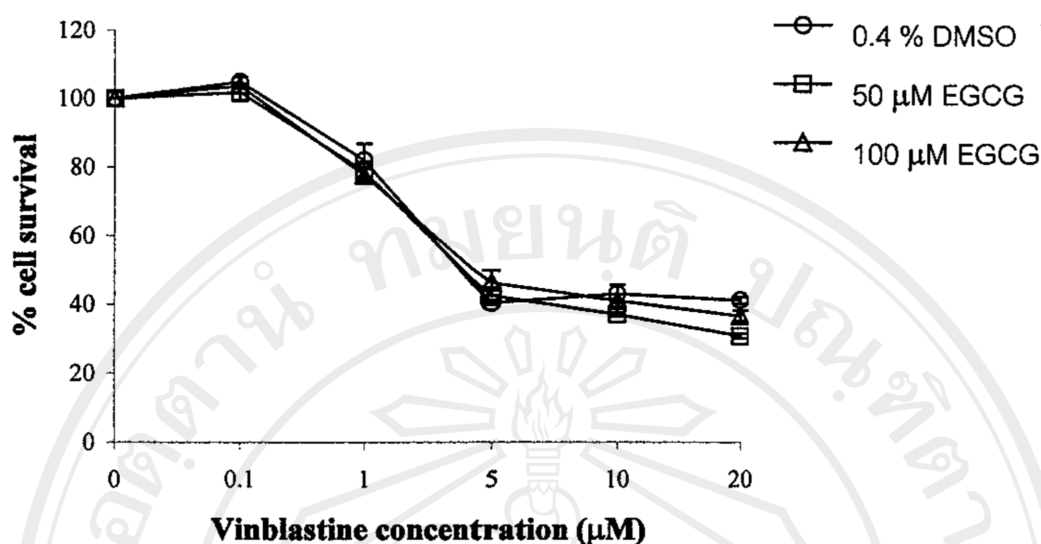

Figure 44. Effect of pre-incubation of EGCG on vinblastine cytotoxicity in KB-V1 cells. KB-V1 cells were pre-incubated with 0.4%DMSO as vehicle control, 50 and 100 μM EGCG. After 48 h incubation, the cells were washed and then incubated with the increasing concentrations of vinblastine without EGCG, then the number of viable cells were determined by MTT assay. Each point represented the mean value of three independent experiments performed in triplicate.

Table 30. Effect of pre-incubation of EGCG on vinblastine cytotoxicity in KB-V1 cells. The data showed in Figure 44 represented mean ± standard deviation of three independent experiments performed in triplicate.

vinblastine concentration (μM)	% cell survival		
	0.4%DMSO	50 μM EGCG	100 μM EGCG
0	100 ± 0	100 ± 0	100 ± 0
0.1	105 ± 1	102 ± 3	104 ± 1
1	82 ± 5	79 ± 3	78 ± 2
5	40 ± 1	42 ± 0	46 ± 4
10	43 ± 3	37 ± 1	41 ± 5
20	41 ± 1	31 ± 1	37 ± 1

*Asterisks denoted values that were significantly different from the vehicle control (0.4% DMSO) at each vinblastine concentration ($P<0.05$).

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