CHAPTER IV
RESULTS

In Vitro-Results

The apoptosis induced by methomyl was shown with different sensitivities in leukocytic cell lines. After 6 hour-exposure, methomyl could reduce mitochondrial transmembrane potential ($\Delta\Psi m$) and induce apoptosis at concentrations of 18 mM (MM6), 12 mM (THP-1), and 12 mM (Jurkat). Number of cells in sub-G_1 area was also detected increased in these cell lines.

Similarly, after 24 hour-exposure, methomyl could reduce $\Delta\Psi m$, increase cell number in sub-G_1 area, and induce apoptosis in these cell lines, however, at lower concentrations: 12 mM (MM6), 6 mM (THP-1), and 6 mM (Jurkat).

Methomyl was not shown to induce apoptosis in Raji cells and DNA fragmentation was not detected, instead, the Raji cells showed a cell cycle arrest in the G_0/G_1 phase.

The apoptosis induced in MM6, THP-1, and Jurkat could be blocked by IL-6. In addition, the apoptosis induced by methomyl was a caspase dependent process since the immunoblot showed a caspase cleavage in Jurkat cells and zVAD-fmk could block methomyl-induced apoptosis in THP-1 cells.

Acetonitrile, which is a metabolite of methomyl, was shown to induce cell death in all cell lines tested. Flow cytometric analysis using annexin V-FITC, TMRE, and PI for cell cycle profile analysis indicated a significant increase of cell death of MM6, THP-1, and Jurkat cells at 5% acetonitrile, while apoptosis of Raji cells was shown at 10% acetonitrile.

Acetonitrile-induced cell death was also shown as a caspase dependent process since the immunoblot of Jurkat cells revealed a caspase cleavage.

Apoptotic Cell Death in MM6, THP-1, Jurkat, and Raji Cells Detected by Annexin V-FITC

Methomyl Exposure for 6 Hours:

Table 4 MM6 cell death detected by annexin V-FITC after methomyl exposure for 6 hours. (* $p<0.05$, compared to control)

Methomyl	% Cell Death (Mean ± S.E.)
0 (Control)	8.41 ± 5.023
0.2	12.04 ± 4.512
0.4	11.50 ± 3.419
0.8	9.55 ± 3.968
1.5	11.21 ± 3.79
3	10.59 ± 2.991
6	12.69 ± 4.743
12	12.96 ± 3.02
18	38.13 ± 9.957*
30	85.89 ± 3.169*

Table 5 THP-1 cell death detected by annexin V-FITC after methomyl exposure for 6 hours. (* p<0.05, compared to control)

Methomyl	% Cell Death (Mean ± S.E.)
0 (Control)	9.67 ± 7.21
0.2	11.40 ± 5.195
0.4	12.23 ± 6.813
0.8	9.97 ± 7.029
1.5	13.74 ± 4.976
3	11.31 ± 6.983
6	14.59 ± 6.13
12	23.58 ± 5.239*
18	65.71 ± 6.93*
30	76.49 ± 11.67*

Table 6 Jurkat cell death detected by annexin V-FITC after methomyl exposure for 6 hours. (* p<0.05, compared to control)

Methomyl	% Cell Death (Mean ± S.E.)
0 (Control)	9.15 ± 5.121
0.2	13.17 ± 2.951
0.4	11.42 ± 3.243
0.8	10.97 ± 2.111
1.5	13.65 ± 4.503
3	9.89 ± 5.007
6	13.86 ± 4.982
12	33.76 ± 9.402*
18	86.39 ± 5.019*
30	93.47 ± 4.901*

Table 7 Raji cell death detected by annexin V-FITC after methomyl exposure for 6 hours.

Methomyl	% Cell Death (Mean ± S.E.)
0 (Control)	14.57 ± 4.798
0.2	13.58 ± 4.727
0.4	14.12 ± 4.560
0.8	13.62 ± 5.248
1.5	12.43 ± 4.843
3	16.02 ± 6.34
6	16.79 ± 6.113
12	17.57 ± 6.901
18	19.79 ± 4.318
30	20.09 ± 5.741

Methomyl Exposure for 24 Hours:

Table 8 MM6 cell death detected by annexin V-FITC after methomyl exposure for 24 hours. (* $p<0.05$, compared to control)

Methomyl	% Cell Death (Mean ± S.E.)
0 (Control)	6.19 ± 5.931
0.2	9.41 ± 5.112
0.4	7.09 ± 6.145
0.8	6.65 ± 4.93
1.5	9.19 ± 6.091
3	10.39 ± 4.018
6	12.16 ± 4.831
12	29.98 ± 4.082*
18	57.83 ± 8.799*
30	88.51 ± 3.97*

Table 9 THP-1 cell death detected by annexin V-FITC after methomyl exposure for 24 hours. (* p<0.05, compared to control)

Methomyl	% Cell Death (Mean ± S.E.)
0 (Control)	8.78 ± 5.321
0.2	10.05 ± 5.458
0.4	9.38 ± 4.96
0.8	11.17 ± 6.239
1.5	9.97 ± 5.682
3	12.11 ± 6.081
6	24.09 ± 5.931*
12	35.83 ± 5.25*
18	76.11 ± 6.519*
30	89.56 ± 5.978*

Table 10 Jurkat cell death detected by annexin V-FITC after methomyl exposure for 24 hours. (* p<0.05, compared to control)

Methomyl	% Cell Death (Mean ± S.E.)
0 (Control)	7.52 ± 6.291
0.2	7.79 ± 5.16
0.4	10.03 ± 6.893
0.8	9.76 ± 4.157
1.5	8.98 ± 6.851
3	11.12 ± 4.93
6	23.66 ± 5.89*
12	47.61 ± 7.093*
18	91.08 ± 5.149*
30	97.73 ± 4.02*

Table 11 Raji cell death detected by annexin V-FITC after methomyl exposure for 24 hours.

Methomyl	% Cell Death (Mean ± S.E.)
0 (Control)	11.72 ± 3.689
0.2	12.41 ± 3.92
0.4	11.94 ± 4.178
0.8	12.05 ± 3.477
1.5	12.93 ± 2.591
3	13.91 ± 4.091
6	10.99 ± 2.97
12	14.08 ± 5.692
18	13.87 ± 4.982
30	16.14 ± 5.483

Methomyl Exposure for 6 Hours:

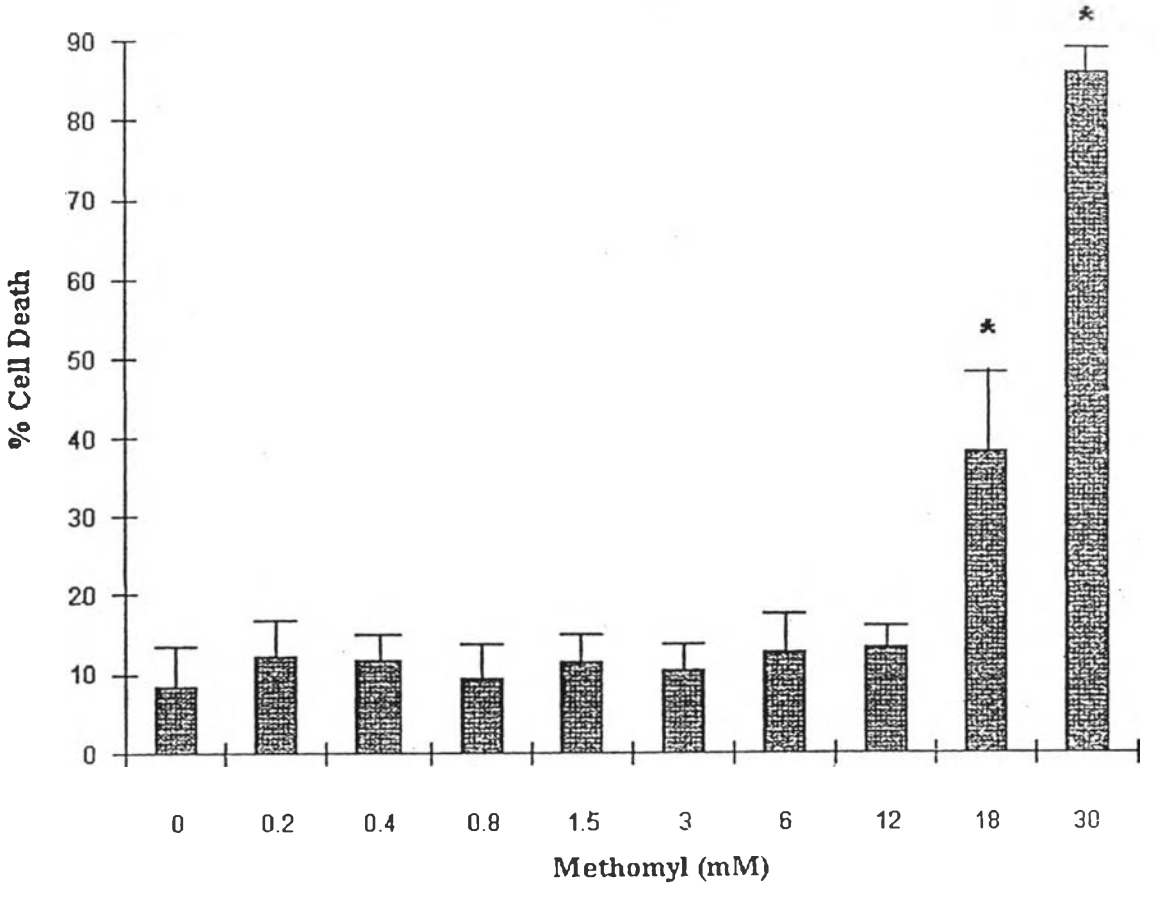

Figure 20 Graph shows percentage of MM6 cell death detected by annexin V-FITC after methomyl exposure for 6 hours. (* $p<0.05$)

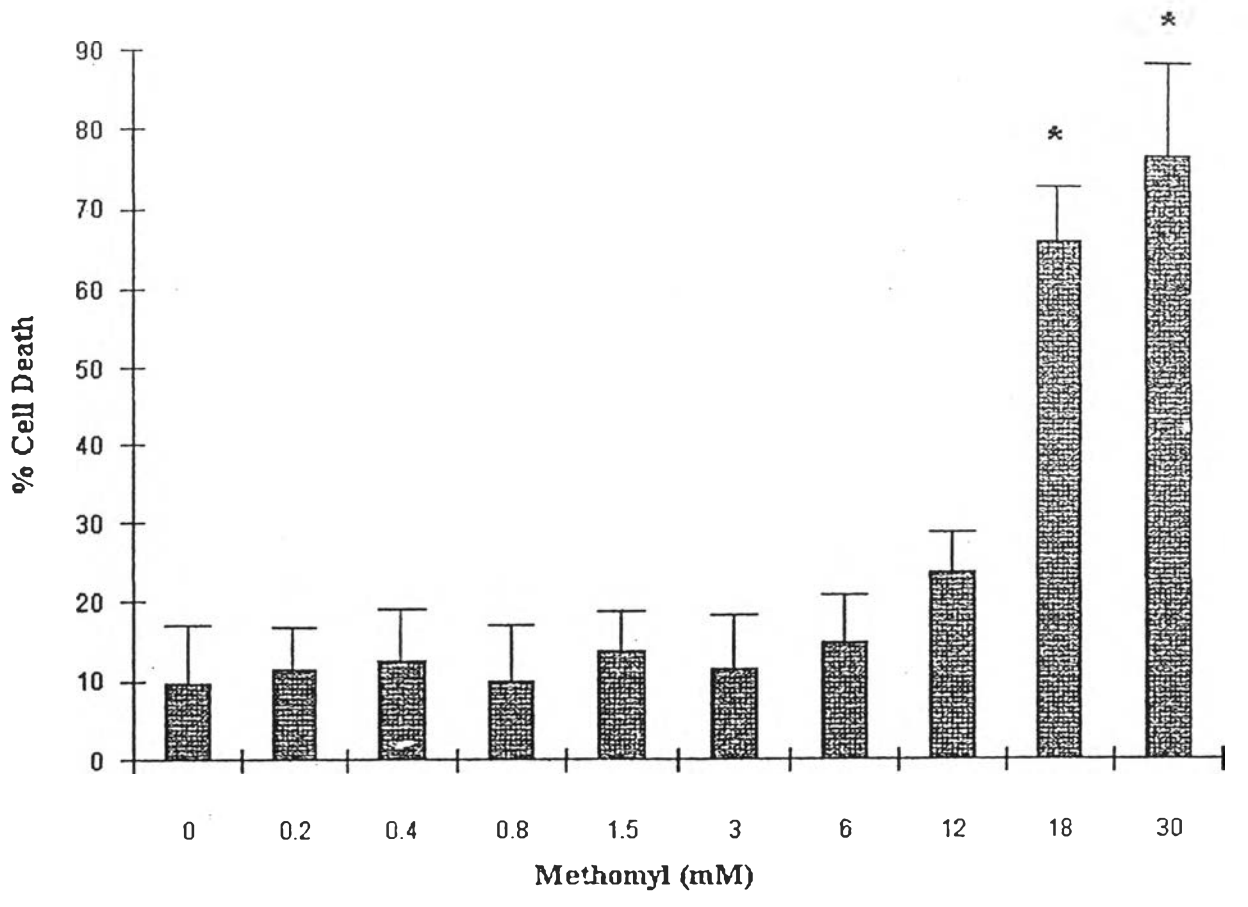

Figure 21 Graph shows percentage of THP-1 cell death detected by annexin V-FITC after methomyl exposure for 6 hours. (* $p<0.05$)

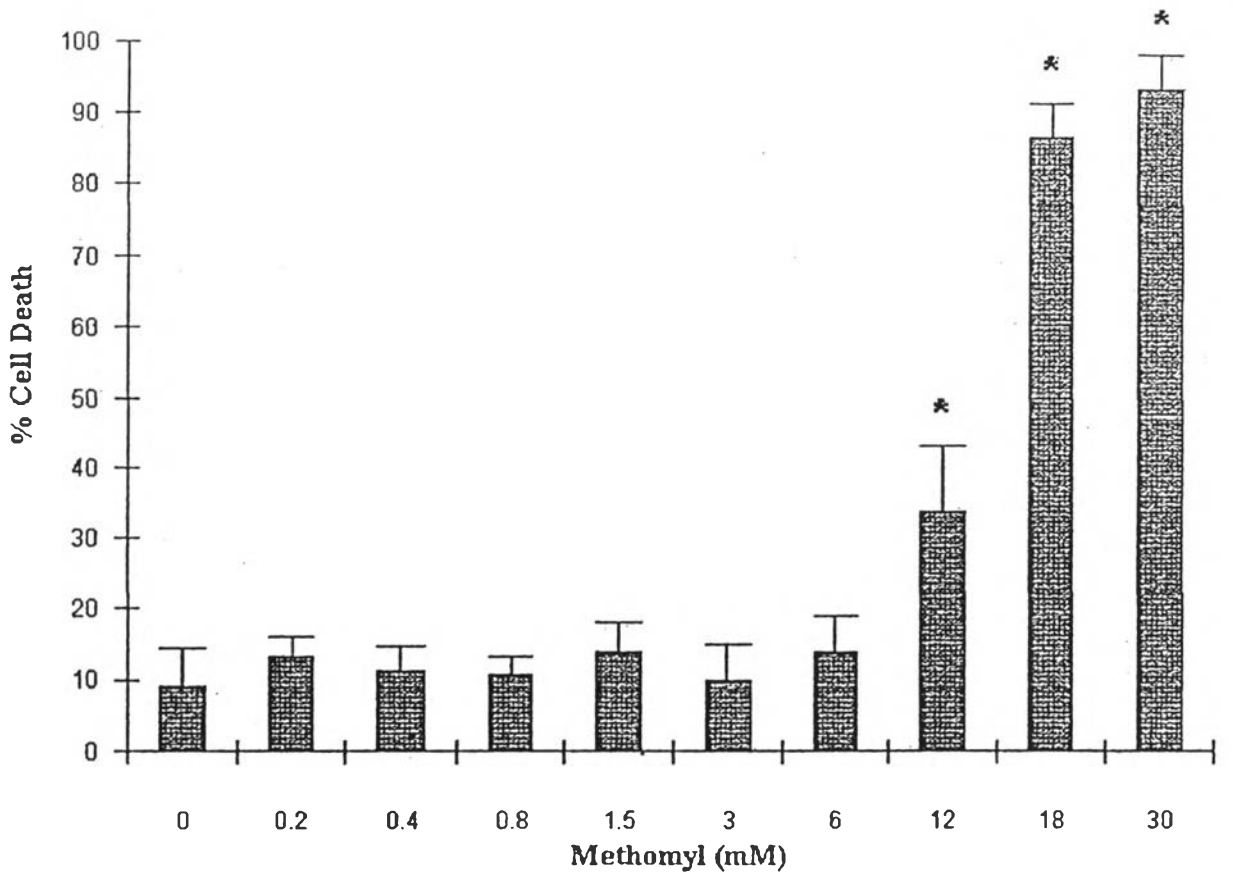

Figure 22 Graph shows percentage of Jurkat cell death detected by annexin V-FITC after methomyl exposure for 6 hours. (* $p<0.05$)

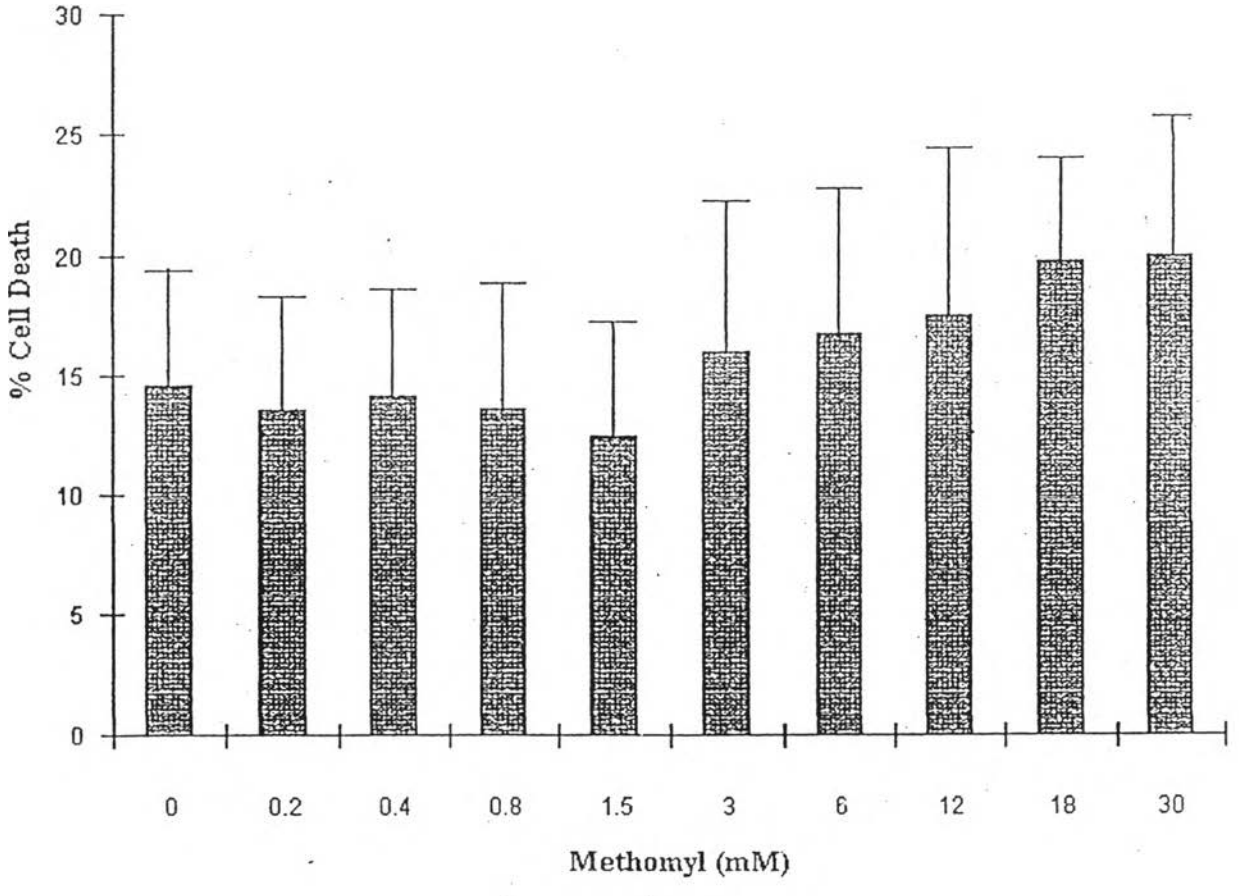

Figure 23 Graph shows percentage of Raji cell death detected by annexin V-FITC after methomyl exposure for 6 hours.

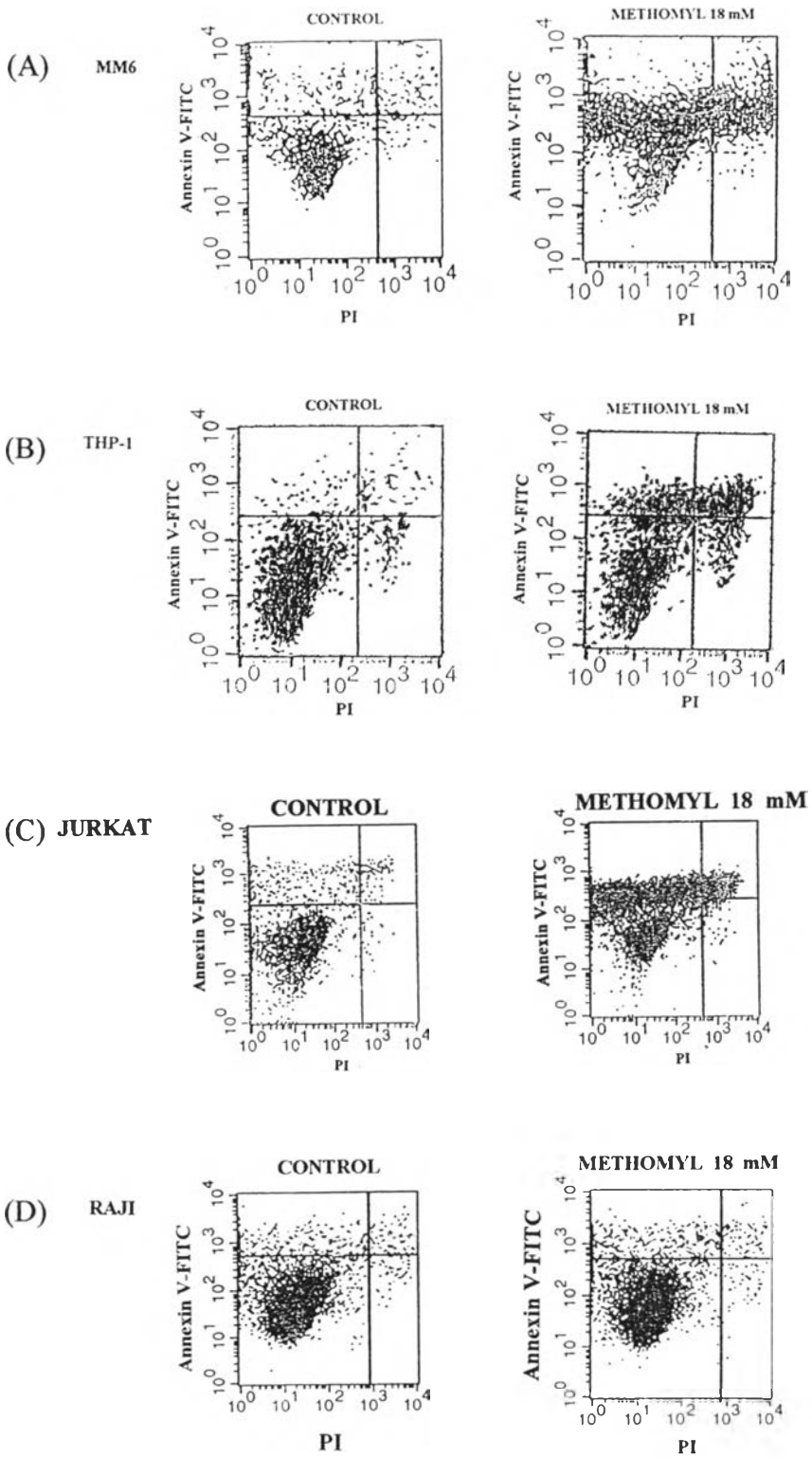

Figure 24 Flow cytometric analysis of cell death detected by annexin V-FITC after methomyl exposure for 6 hours. (A) MM6, (B) THP-1, (C) Jurkat, and (D) Raji.

The Reduction of Mitrochondrial Transmembrane Potential in MM6, THP-1, Jurkat, and Raji Cells Detected by TMRE

Methomyl Exposure for 6 Hours:

Table 12 Reduction of mitochondrial transmembrane potential in MM6 cells detected by TMRE after methomyl exposure for 6 hours. (* $p<0.05$, compared to control)

Methomyl	% Cells with $\downarrow\Delta\Psi$ (Mean ± S.E.)
0 (Control)	10.94 ± 2.46
0.2	10.18 ± 1.785
0.4	14.25 ± 6.178
0.8	12.88 ± 2.52
1.5	11.36 ± 3.401
3	13.21 ± 3.759
6	14.77 ± 4.591
12	15.06 ± 5.134
18	47.07 ± 13.18*
30	89.45 ± 3.029*

Table 13 Reduction of mitochondrial transmembrane potential in THP-1 cells detected by TMRE after methomyl exposure for 6 hours. (* p<0.05, compared to control)

Methomyl	% Cells with $\downarrow\Delta\Psi$ (Mean ± S.E.)
0 (Control)	12.58 ± 5.82
0.2	14.10 ± 6.531
0.4	12.02 ± 3.78
0.8	11.99 ± 4.932
1.5	15.67 ± 7.089
3	14.91 ± 6.504
6	18.99 ± 6.656
12	27.28 ± 5.562*
18	67.83 ± 7.21*
30	74.34 ± 15.33*

Table 14 Reduction of mitochondrial transmembrane potential in Jurkat cells detected by TMRE after methomyl exposure for 6 hours. (* p<0.05, compared to control)

Methomyl	% Cells with $\downarrow\Delta\Psi$ (Mean ± S.E.)
0 (Control)	11.08 ± 3.554
0.2	9.01 ± 2.824
0.4	11.68 ± 4.031
0.8	13.15 ± 2.609
1.5	12.16 ± 5.856
3	12.78 ± 2.893
6	14.01 ± 5.185
12	42.36 ± 8.401*
18	94.03 ± 2.134*
30	96.55 ± 3.311*

Table 15 Reduction of mitochondrial transmembrane potential in Raji cells detected by TMRE after methomyl exposure for 6 hours.

Methomyl	% Cells with $\downarrow\Delta\Psi$ (Mean ± S.E.)
0 (Control)	15.9 ± 5.007
0.2	15.06 ± 2.585
0.4	13.12 ± 3.583
0.8	14.59 ± 2.624
1.5	14.70 ± 3.609
3	13.96 ± 2.573
6	15.53 ± 3.307
12	18.87 ± 5.941
18	20.19 ± 6.238
30	21.02 ± 6.989

Methomyl Exposure for 24 Hours:

Table 16 Reduction of mitochondrial transmembrane potential in MM6 cells detected by TMRE after methomyl exposure for 24 hours. (* $p<0.05$, compared to control)

Methomyl	% Cells with $\downarrow\Delta\Psi$ (Mean ± S.E.)
0 (Control)	8.89 ± 4.619
0.2	10.27 ± 3.257
0.4	10.03 ± 4.43
0.8	8.94 ± 4.213
1.5	9.64 ± 3.981
3	10.19 ± 4.794
6	11.25 ± 4.695
12	23.19 ± 5.87*
18	51.01 ± 6.825*
30	92.63 ± 3.612*

Table 17 Reduction of mitochondrial transmembrane potential in THP-1 cells detected by TMRE after methomyl exposure for 24 hours. (* p<0.05, compared to control)

Methomyl	% Cells with $\downarrow\Delta\Psi$ (Mean ± S.E.)
0 (Control)	10.14 ± 5.21
0.2	10.09 ± 4.932
0.4	11.75 ± 5.047
0.8	9.97 ± 6.112
1.5	10.73 ± 5.282
3	11.95 ± 6.022
6	26.42 ± 6.48*
12	33.12 ± 5.117*
18	74.58 ± 6.191*
30	86.47 ± 7.029*

Table 18 Reduction of mitochondrial transmembrane potential in Jurkat cells detected by TMRE after methomyl exposure for 24 hours. (* p<0.05, compared to control)

Methomyl	% Cells with $\downarrow\Delta\Psi$ (Mean ± S.E.)
0 (Control)	9.14 ± 4.199
0.2	9.12 ± 3.843
0.4	9.97 ± 4.398
0.8	10.06 ± 4.655
1.5	10.11 ± 5.145
3	12.86 ± 6.387
6	22.09 ± 5.228*
12	32.61 ± 6.79*
18	97.18 ± 2.016*
30	98.07 ± 2.97*

Table 19 Reduction of mitochondrial transmembrane potential in Raji cells detected by TMRE after methomyl exposure for 24 hours.

Methomyl	% Cells with $\downarrow\Delta\Psi$ (Mean ± S.E.)
0 (Control)	11.28 ± 3.178
0.2	10.94 ± 2.891
0.4	11.09 ± 3.87
0.8	9.97 ± 3.676
1.5	11.61 ± 3.601
3	11.76 ± 3.51
6	13.22 ± 3.514
12	13.16 ± 4.784
18	16.02 ± 3.988
30	16.97 ± 4.847

Methomyl Exposure for 6 Hours:

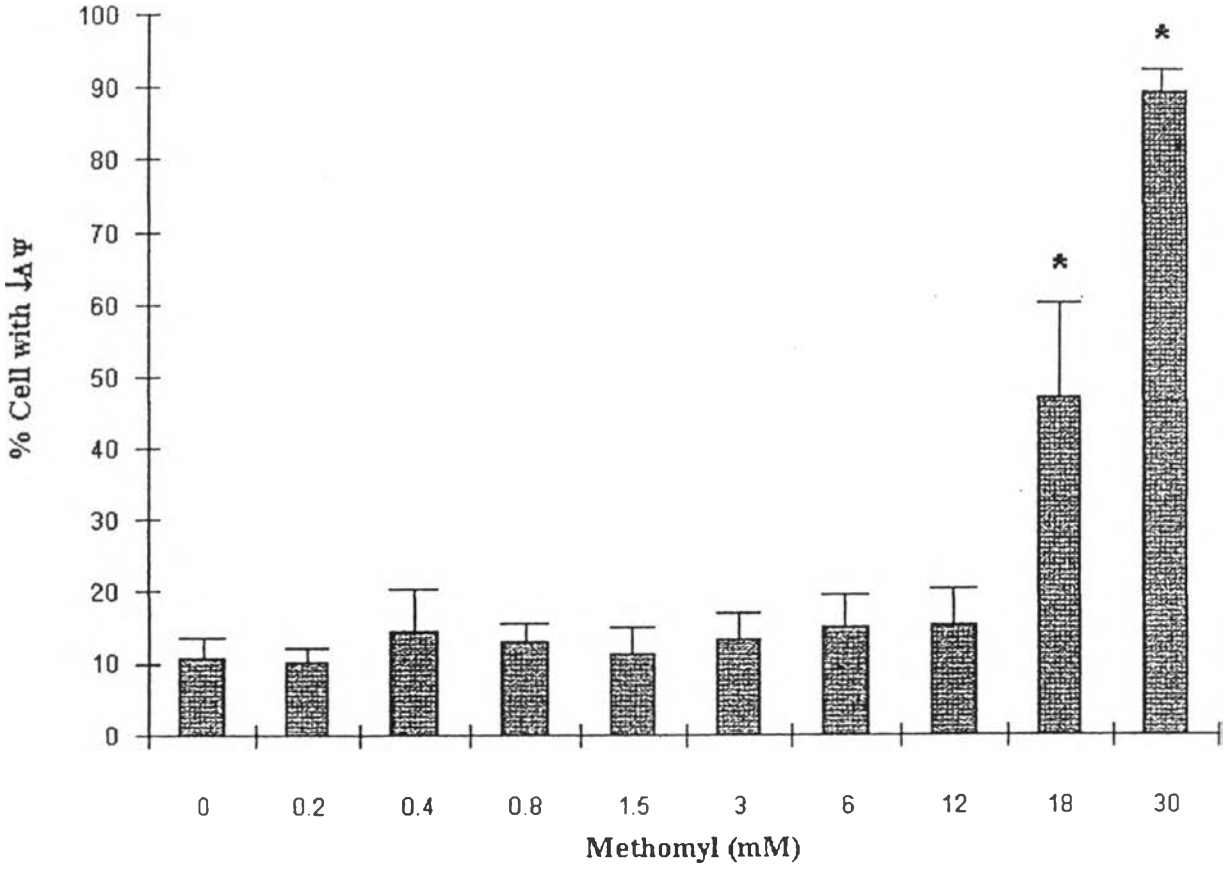

Figure 25 Graph shows percentage of MM6 cells with the reduction of mitochondrial transmembrane potential detected by TMRE after methomyl exposure for 6 hours. (* $p<0.05$)

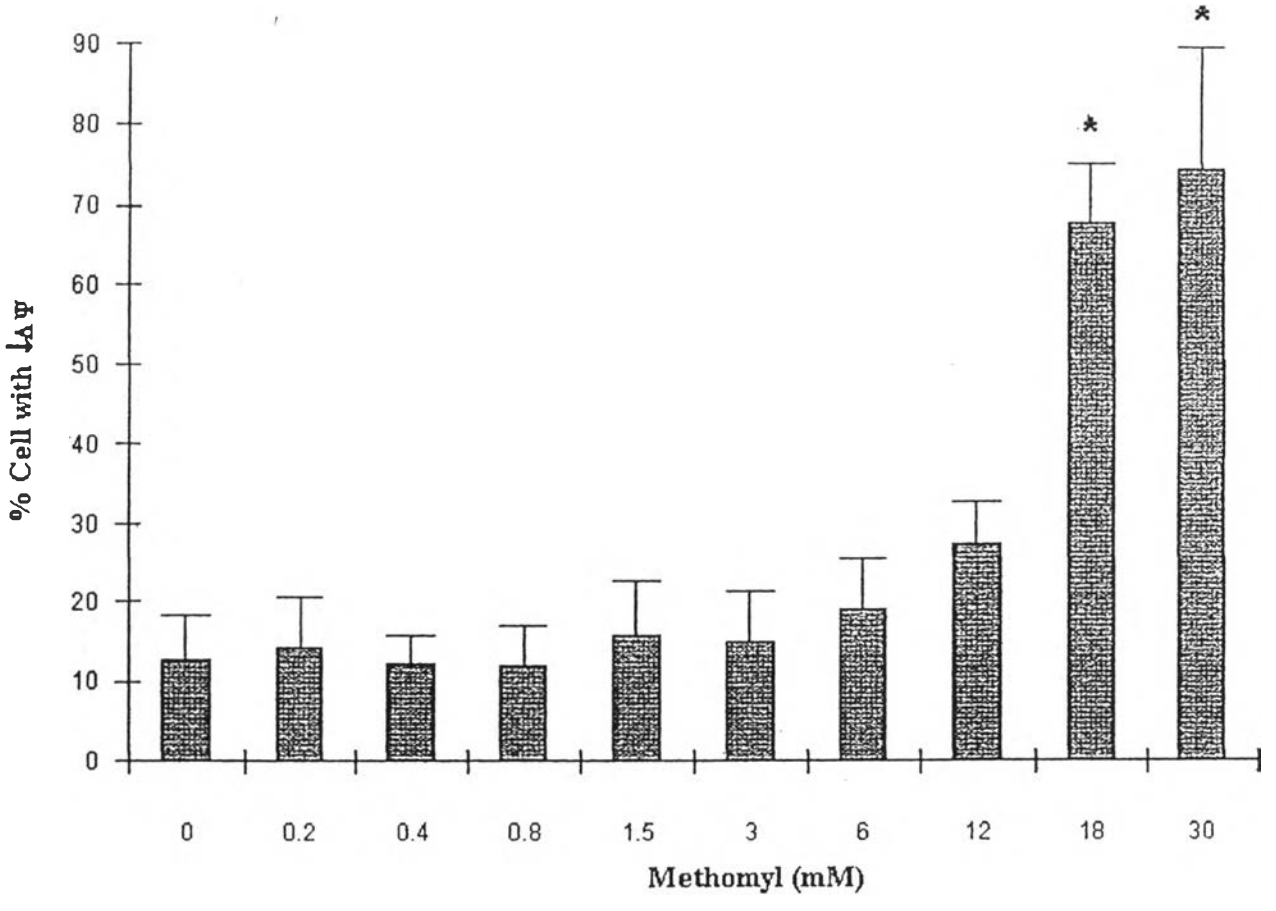

Figure 26 Graph shows percentage of THP-1 cells with the reduction of mitochondrial transmembrane potential detected by TMRE after methomyl exposure for 6 hours. (* $p<0.05$)

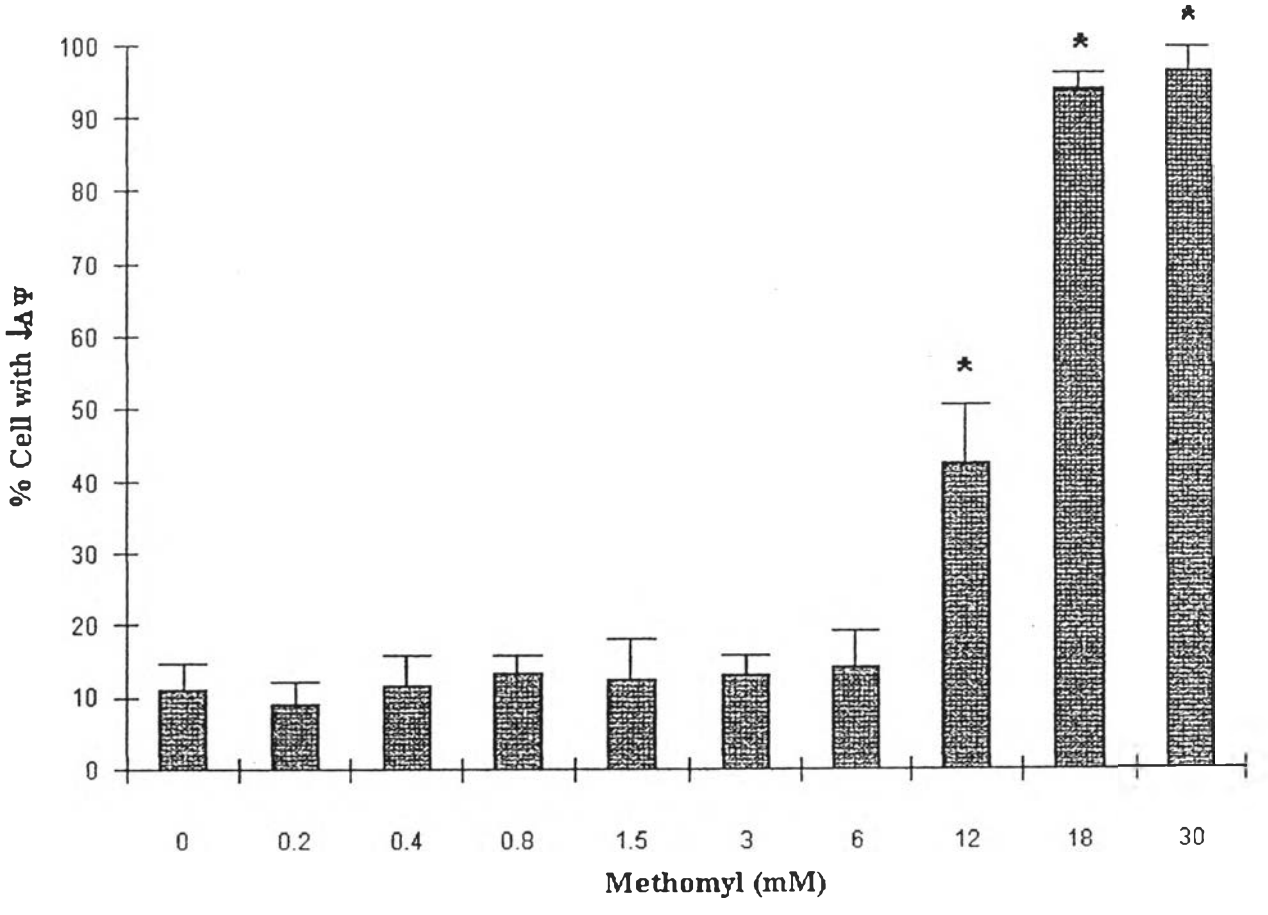

Figure 27 Graph shows percentage of Jurkat cells with the reduction of mitochondrial transmembrane potential detected by TMRE after methomyl exposure for 6 hours. (* $p<0.05$)

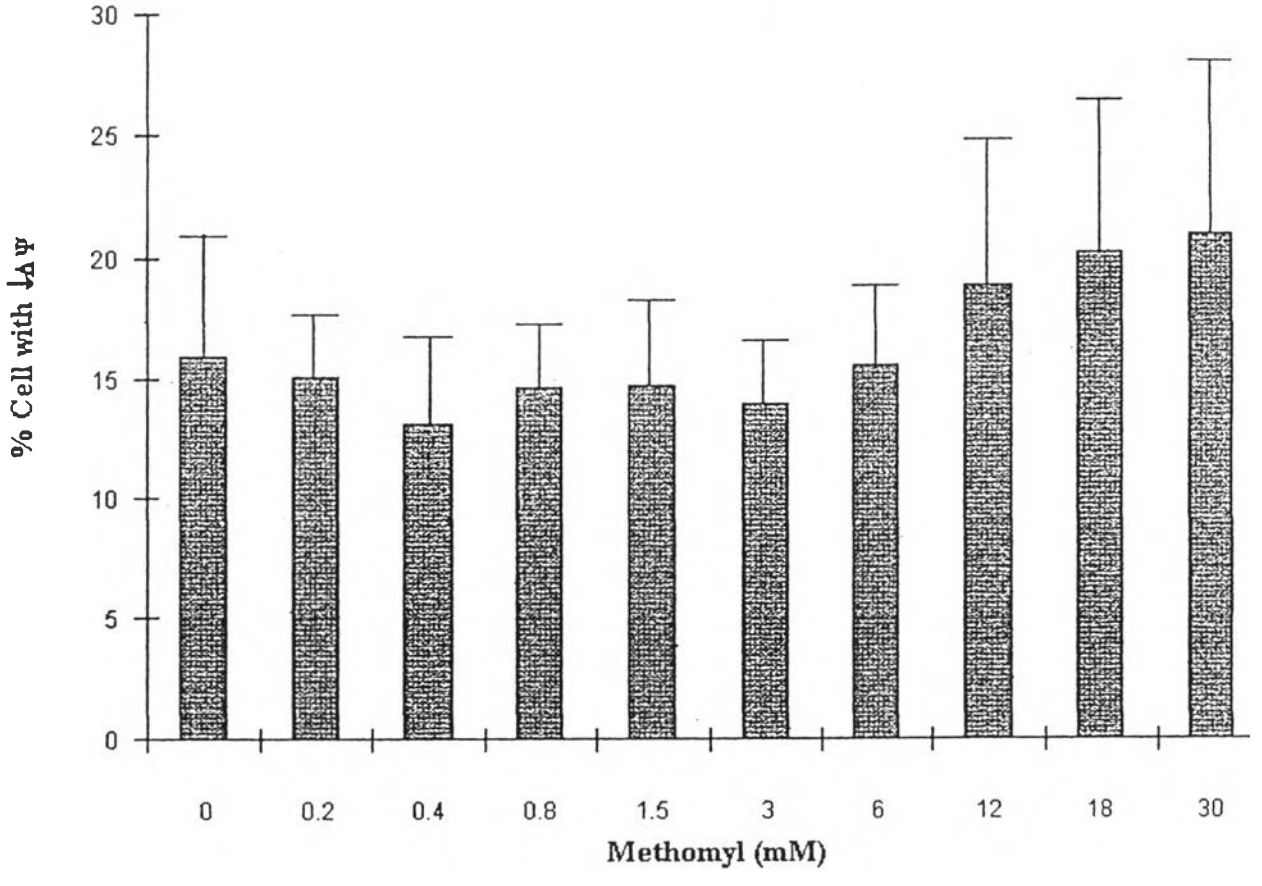

Figure 28 Graph shows percentage of Raji cells with the reduction of mitochondrial transmembrane potential detected by TMRE after methomyl exposure for 6 hours.

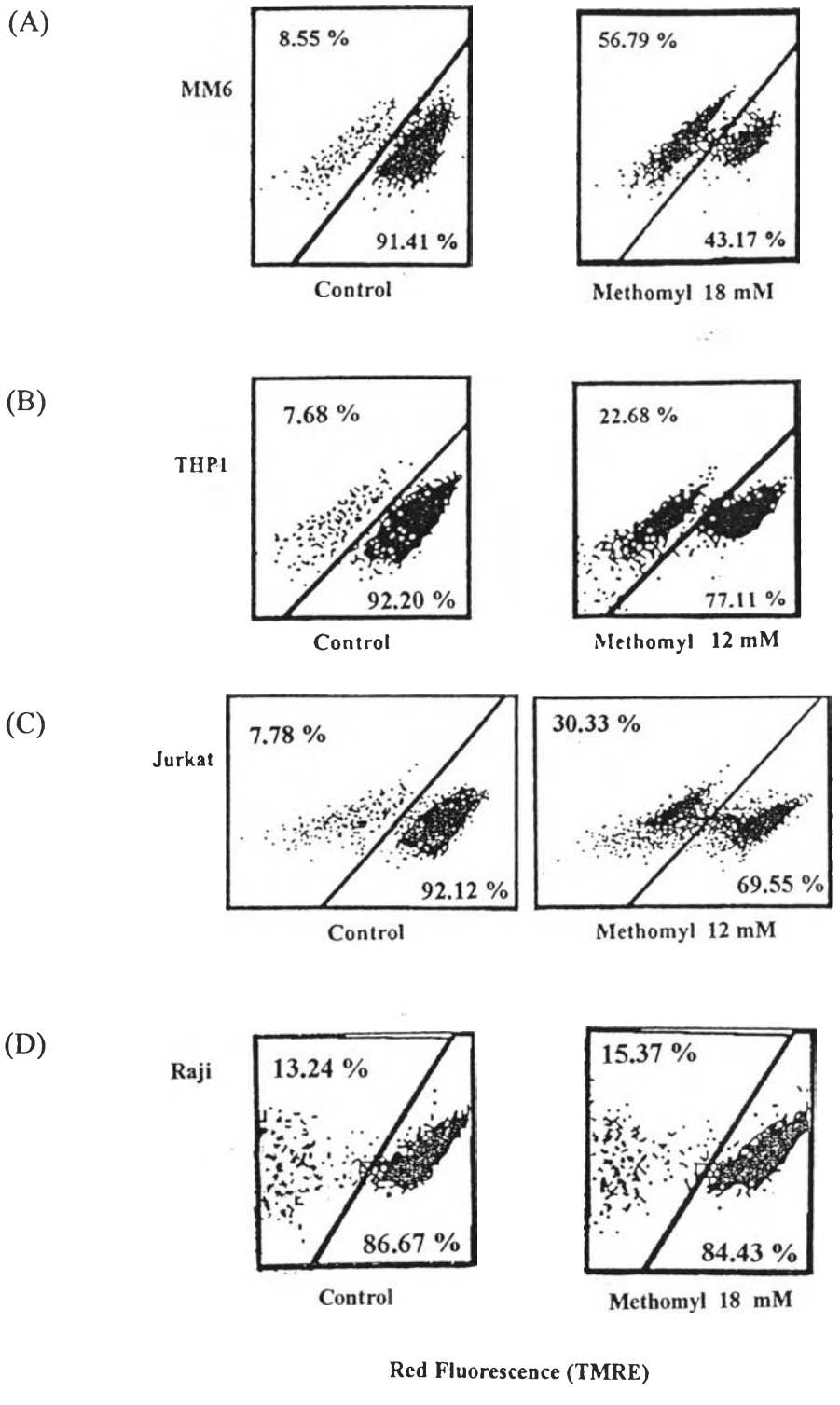

Figure 29 Flow cytometric analysis of the reduction of mitochondrial transmembrane potential detected by TMRE after methomyl exposure for 6 hours. (A) MM6, (B), THP-1, (C) Jurkat, and (D) Raji. Cells with the lower red fluorescence (TMRE) (or cells in the left gate) are cells with $\downarrow\Delta\Psi$.

DNA Fragmentation of in MM6, THP-1, Jurkat, and Raji Cells Shown in the Sub-G_1 Area Detected by PI

Methomyl Exposure for 6 Hours:

Table 20 Increase of MM6 cells in the sub-G_1 area detected by PI after methomyl exposure for 6 hours. (* $p<0.05$, compared to control)

Methomyl	% Cells in the Sub-G_1 Area (Mean ± S.E.)
0 (Control)	6.86 ± 2.563
0.2	6.28 ± 1.803
0.4	6.35 ± 2.034
0.8	7.01 ± 3.115
1.5	7.43 ± 2.41
3	7.12 ± 3.048
6	8.14 ± 2.49
12	10.23 ± 2.523
18	19.87 ± 3.016*
30	48.67 ± 3.274*

Table 21 Increase of THP-1 cells in the sub-G_1 area detected by PI after methomyl exposure for 6 hours. (* $p<0.05$, compared to control)

Methomyl	% Cells in the Sub-G_1 Area (Mean ± S.E.)
0 (Control)	3.57 ± 2.943
0.2	3.12 ± 2.05
0.4	3.81 ± 3.057
0.8	4.09 ± 3.311
1.5	3.76 ± 2.844
3	4.92 ± 3.099
6	6.13 ± 3.841
12	11.85 ± 4.962*
18	23.39 ± 4.831*
30	28.24 ± 5.253*

Table 22 Increase of Jurkat cells in the sub-G_1 area detected by PI after methomyl exposure for 6 hours. (* $p<0.05$, compared to control)

Methomyl	% Cells in the Sub-G_1 Area (Mean ± S.E.)
0 (Control)	3.07 ± 2.98
0.2	2.96 ± 3.159
0.4	3.39 ± 2.763
0.8	3.27 ± 2.87
1.5	3.43 ± 3.015
3	3.72 ± 2.95
6	4.33 ± 2.784
12	13.62 ± 4.606*
18	49.89 ± 5.109*
30	58.13 ± 4.81*

Table 23 Increase of Raji cells in the sub-G_1 area detected by PI after methomyl exposure for 6 hours.

Methomyl	% Cells in the Sub-G_1 Area (Mean ± S.E.)
0 (Control)	4.51 ± 1.15
0.2	4.36 ± 1.438
0.4	4.54 ± 1.59
0.8	4.51 ± 2.012
1.5	4.72 ± 1.693
3	4.61 ± 1.717
6	4.48 ± 2.11
12	4.67 ± 1.644
18	4.43 ± 1.249
30	4.75 ± 2.16

Methomyl Exposure for 24 Hours:

Table 24 Increase of MM6 cells in the sub-G_1 area detected by PI after methomyl exposure for 24 hours. (* $p<0.05$, compared to control)

Methomyl	% Cells in the Sub-G_1 Area (Mean ± S.E.)
0 (Control)	7.28 ± 2.689
0.2	7.12 ± 2.56
0.4	7.43 ± 2.637
0.8	7.81 ± 2.654
1.5	7.86 ± 2.199
3	8.25 ± 3.812
6	9.14 ± 2.392
12	15.33 ± 3.017*
18	23.79 ± 3.194*
30	59.79 ± 3.89*

Table 25 Increase of THP-1 cells in the sub-G_1 area detected by PI after methomyl exposure for 24 hours. (* $p<0.05$, compared to control)

Methomyl	% Cells in the Sub-G_1 Area (Mean ± S.E.)
0 (Control)	4.68 ± 2.897
0.2	4.91 ± 2.591
0.4	5.11 ± 2.88
0.8	4.97 ± 3.115
1.5	5.68 ± 2.923
3	7.13 ± 3.139
6	11.43 ± 3.23*
12	17.54 ± 3.944*
18	29.9 ± 4.831*
30	30.26 ± 4.253*

Table 26 Increase of Jurkat cells in the sub-G_1 area detected by PI after methomyl exposure for 24 hours. (* $p<0.05$, compared to control)

Methomyl	% Cells in the Sub-G_1 Area (Mean ± S.E.)
0 (Control)	3.79 ± 3.072
0.2	3.24 ± 2.941
0.4	3.97 ± 2.879
0.8	3.76 ± 3.451
1.5	4.31 ± 3.705
3	4.56 ± 3.82
6	10.67 ± 3.17*
12	17.41 ± 3.874*
18	52.08 ± 4.053*
30	60.37 ± 3.986*

Table 27 Increase of Raji cells in the sub-G_1 area detected by PI after methomyl exposure for 24 hours.

Methomyl	% Cells in the Sub-G_1 Area (Mean ± S.E.)
0 (Control)	4.75 ± 2.019
0.2	4.51 ± 1.841
0.4	4.67 ± 1.953
0.8	4.71 ± 1.63
1.5	4.68 ± 2.102
3	4.84 ± 2.05
6	5.13 ± 2.87
12	4.77 ± 1.918
18	4.94 ± 2.167
30	4.49 ± 2.655

Methomyl Exposure for 6 Hours:

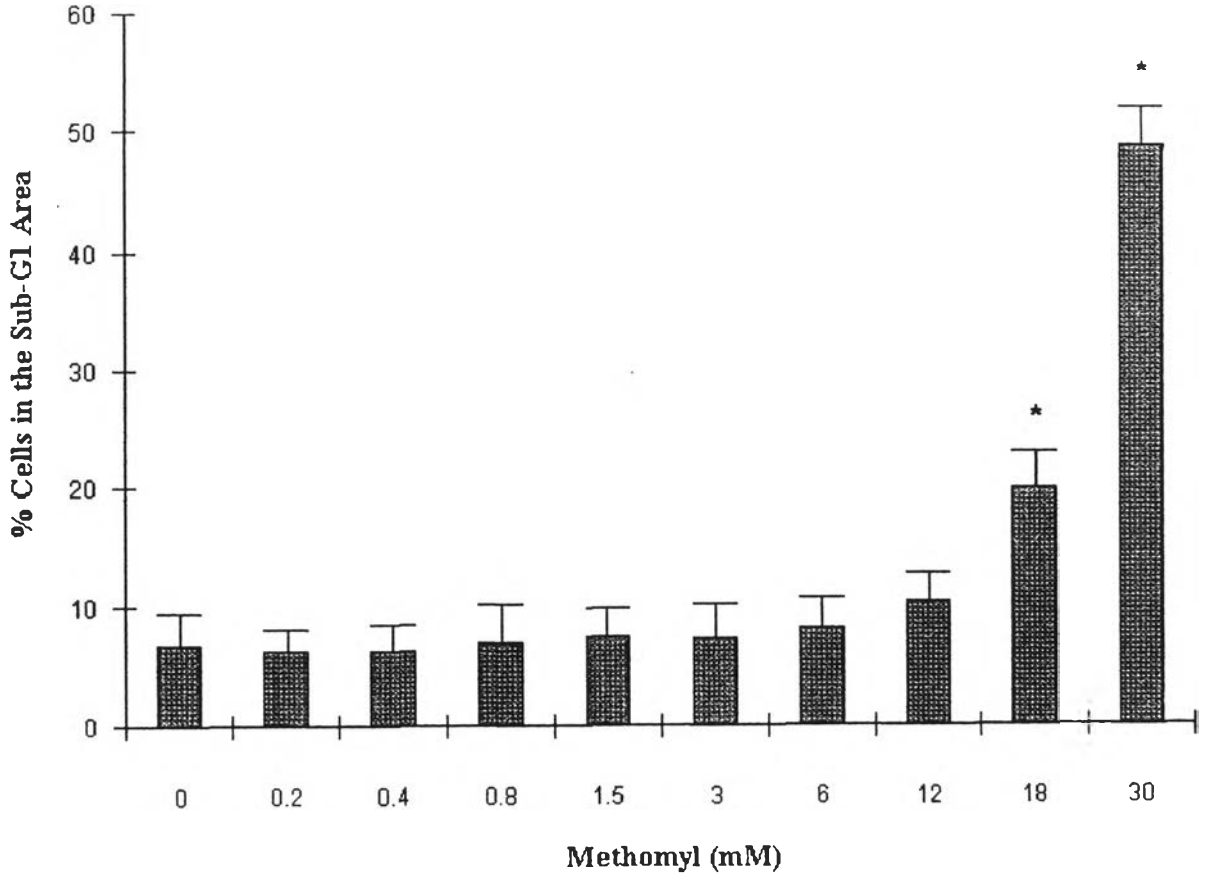

Figure 30 Graph shows percentage of MM6 cells in the sub-G_1 area detected by PI after methomyl exposure for 6 hours. (* $p<0.05$)

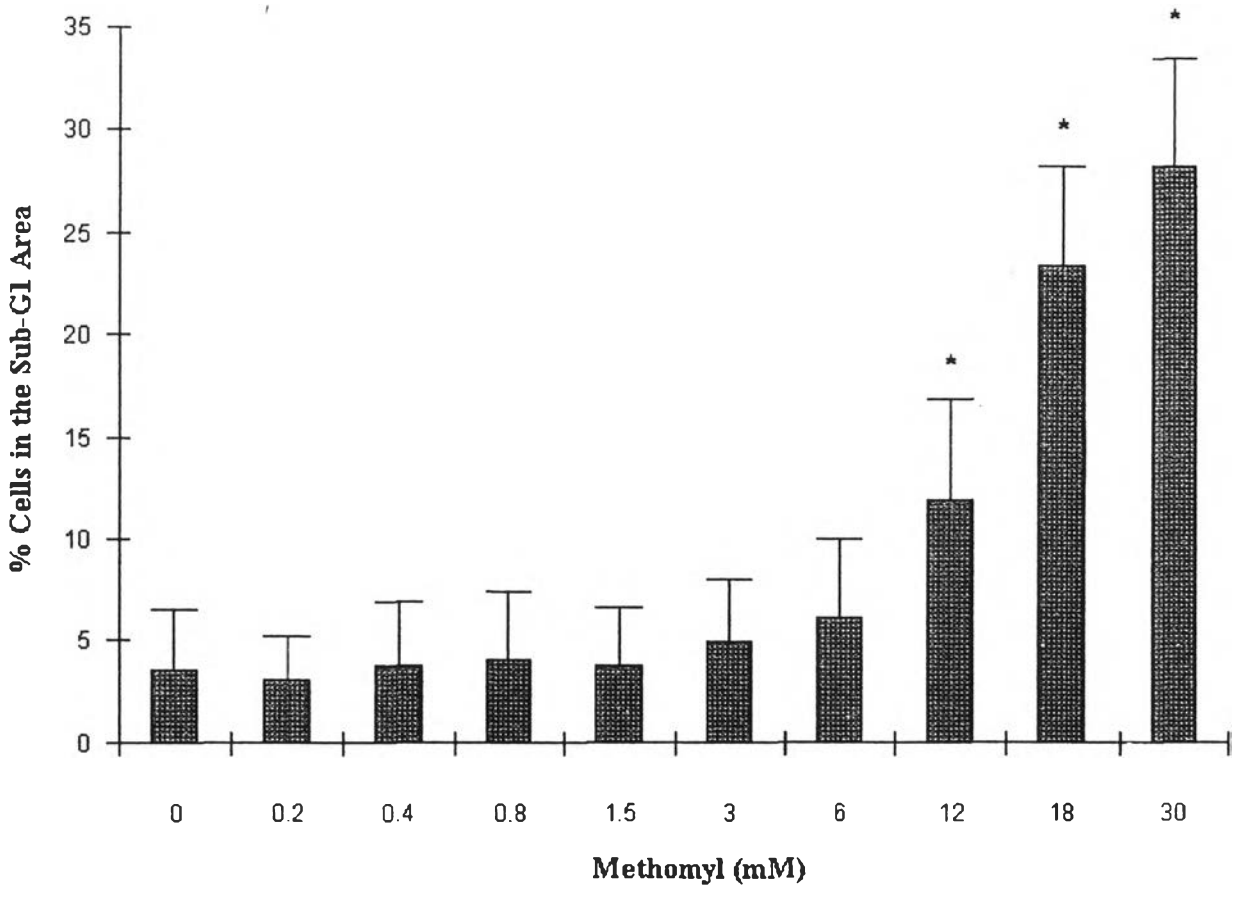

Figure 31 Graph shows percentage of THP-1 cells in the sub-G_1 area detected by PI after methomyl exposure for 6 hours. (* $p<0.05$)

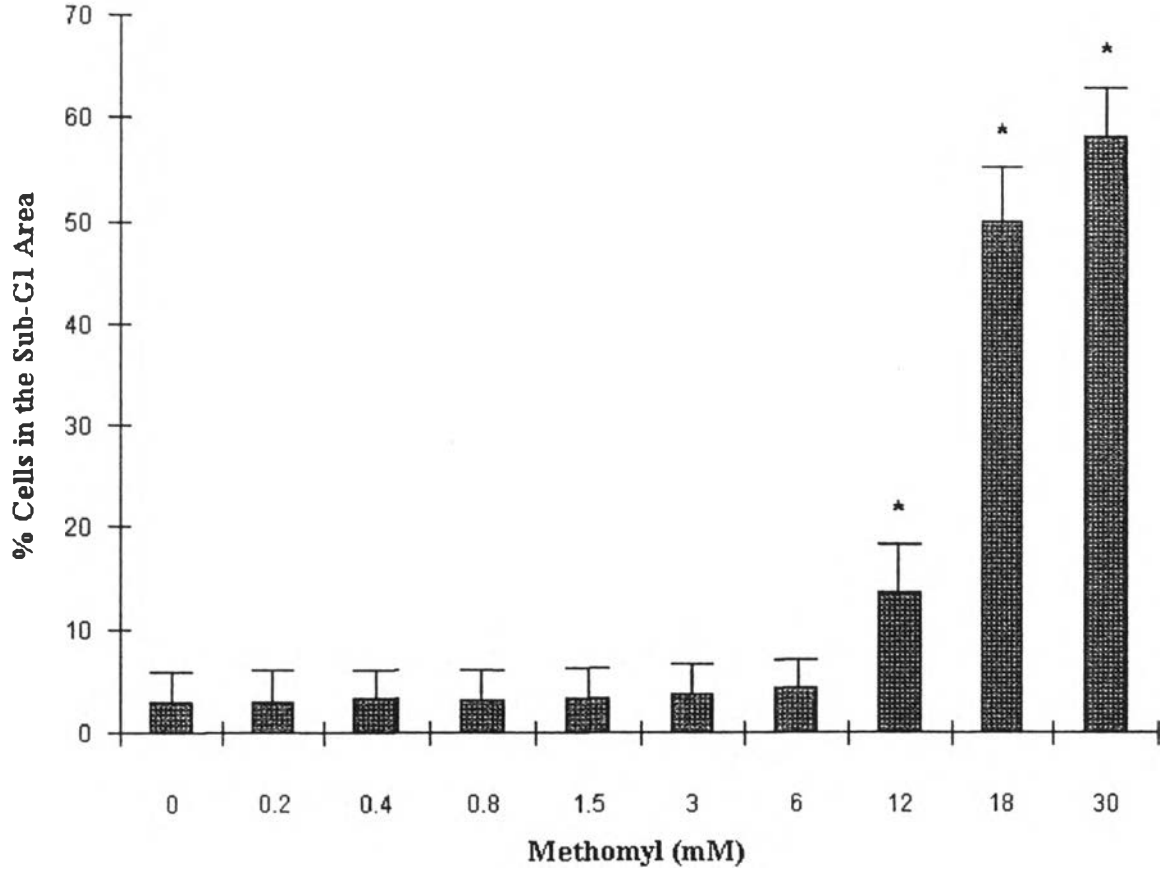

Figure 32 Graph shows percentage of Jurkat cells in the sub-G_1 area detected by PI after methomyl exposure for 6 hours. (* $p<0.05$)

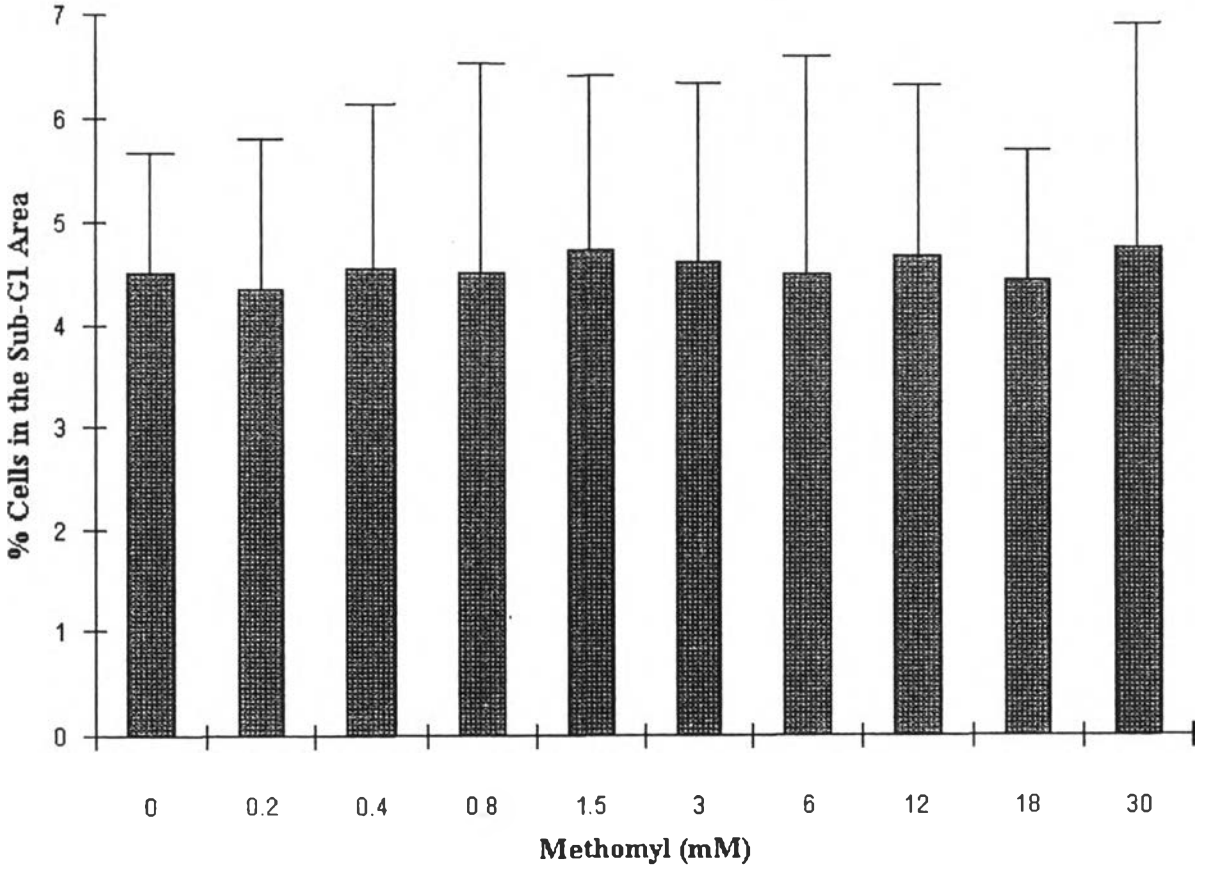

Figure 33 Graph shows percentage of Raji cells in the sub-G_1 area detected by PI after methomyl exposure for 6 hours.

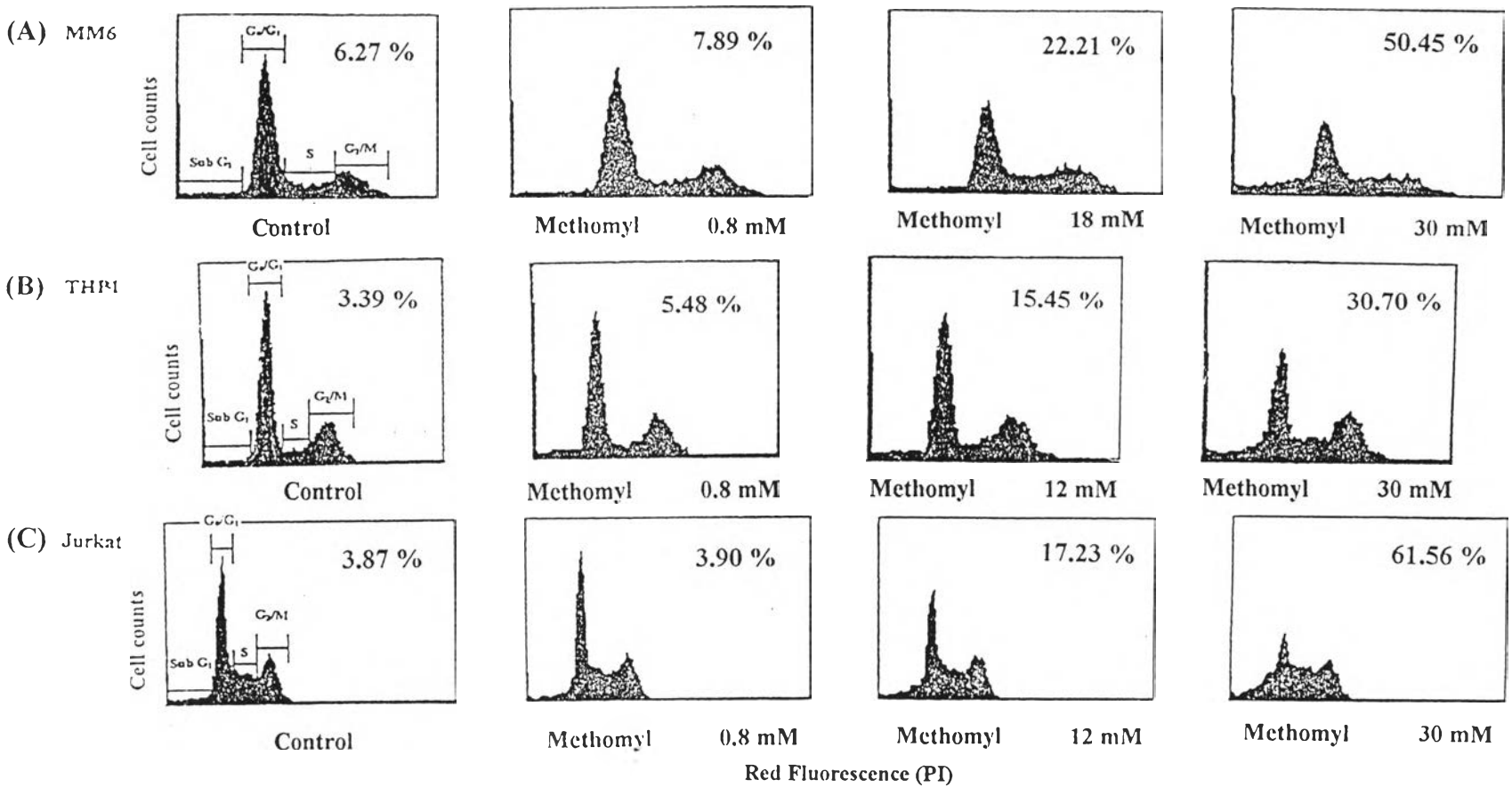

Figure 34 Flow cytometric analysis of cells with DNA fragmentation in the sub-G_1 area detected by PI after methomyl exposure for 6 hours. (A) MM6, (B), THP-1, and (C) Jurkat. The increases in percentage of cells in the sub-G_1 area are shown.

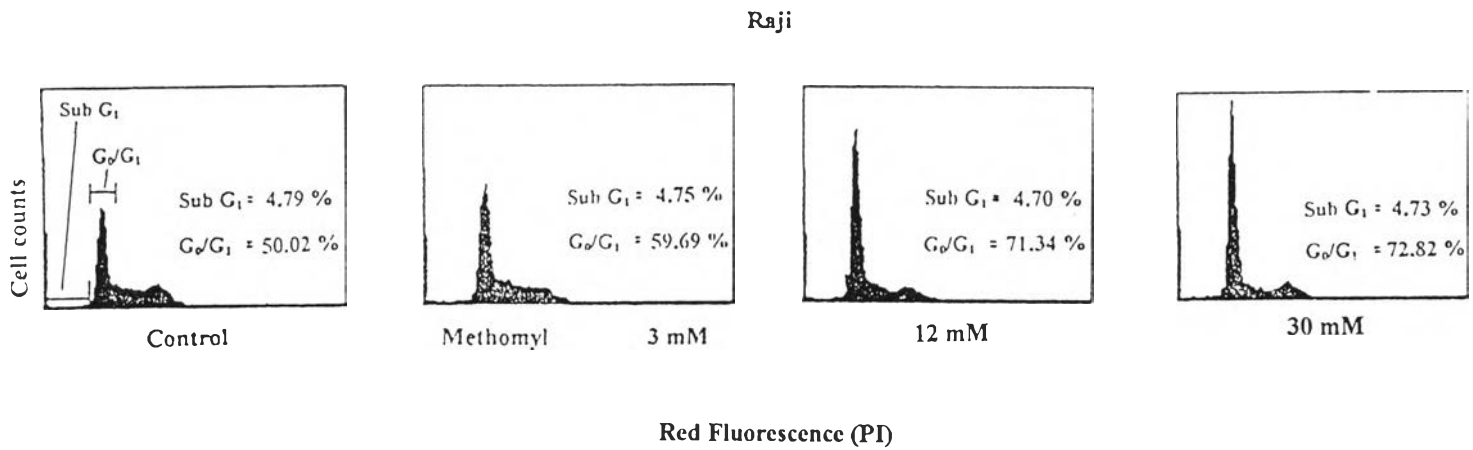

Figure 35 Flow cytometric analysis of the DNA fragmentation in Raji cells detected by PI after methomyl exposure for 6 hours. The cell cycle profile shows cell cycle arrest in the G_0/G_1 phase.

Cell Death in MM6, THP-1, Jurkat, and Raji Cells Detected by Annexin V-FITC

Acetonitrile Exposure for 6 Hours:

Table 28 MM6 cell death detected by annexin V-FITC after acetonitrile exposure for 6 hours. (* p<0.05, compared to control)

% Acetonitrile	% Cell Death (Mean ± S.E.)
0 (control)	12.13 ± 6.1
0.1	11.38 ± 7.71
0.5	7.62 ± 5.28
1	3.48 ± 2.795
5	35.15 ± 10.26*
10	92.86 ± 7.988*
30	99.15 ± 0.596*

Table 29 THP-1 cell death detected by annexin V-FITC after acetonitrile exposure for 6 hours. (* p<0.05, compared to control)

% Acetonitrile	% Cell Death (Mean ± S.E.)
0 (control)	13.39 ± 4.098
0.1	13.05 ± 3.747
0.5	10.35 ± 2.149
1	14.36 ± 2.407
5	46.64 ± 14.82*
10	69.58 ± 18.85*
30	87.69 ± 4.773*

Table 30 Jurkat cell death detected by annexin V-FITC after acetonitrile exposure for 6 hours. (* p<0.05, compared to control)

% Acetonitrile	% Cell Death (Mean ± S.E.)
0 (control)	9.64 ± 6.558
0.1	5.57 ± 2.142
0.5	6.87 ± 3.724
1	4.44 ± 2.952
5	45.39 ± 15.25*
10	86.83 ± 12.75*
30	97.08 ± 1.272*

Table 31 Raji cell death detected by annexin V-FITC after acetonitrile exposure for 6 hours. (* p<0.05, compared to control)

% Acetonitrile	% Cell Death (Mean ± S.E.)
0 (control)	12.23 ± 4.217
0.1	11.81 ± 4.661
0.5	11.01 ± 3.543
1	8.78 ± 6.689
5	13.85 ± 6.444
10	63.71 ± 17.06*
30	83.91 ± 10.48*

Acetonitrile Exposure for 6 Hours:

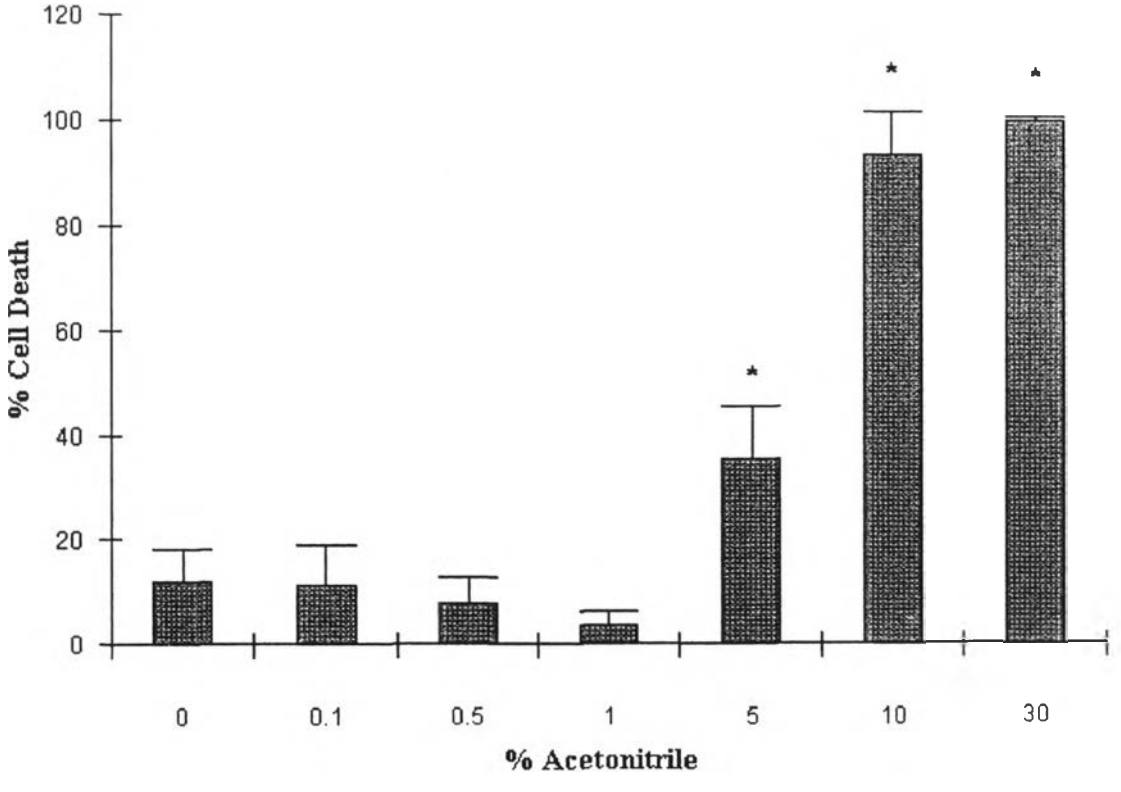

Figure 36 Graph shows percentage of MM6 cell death detected by annexin V-FITC after acetonitrile exposure for 6 hours. (* $p<0.05$)

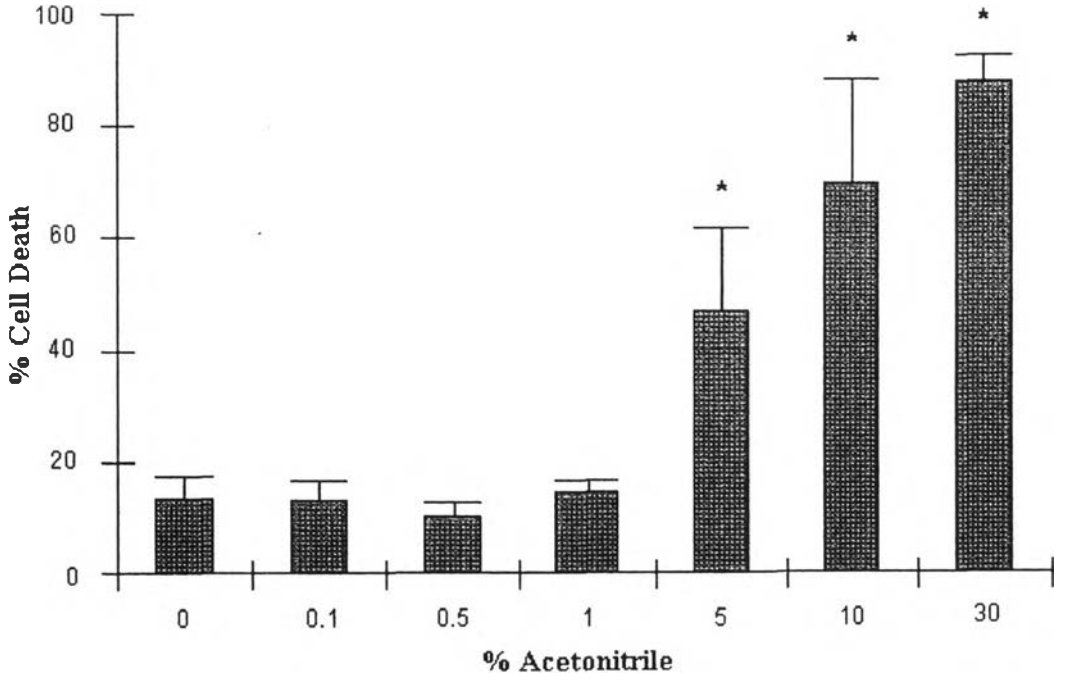

Figure 37 Graph shows percentage of THP-1 cell death detected by annexin V-FITC after acetonitrile exposure for 6 hours. (* $p<0.05$)

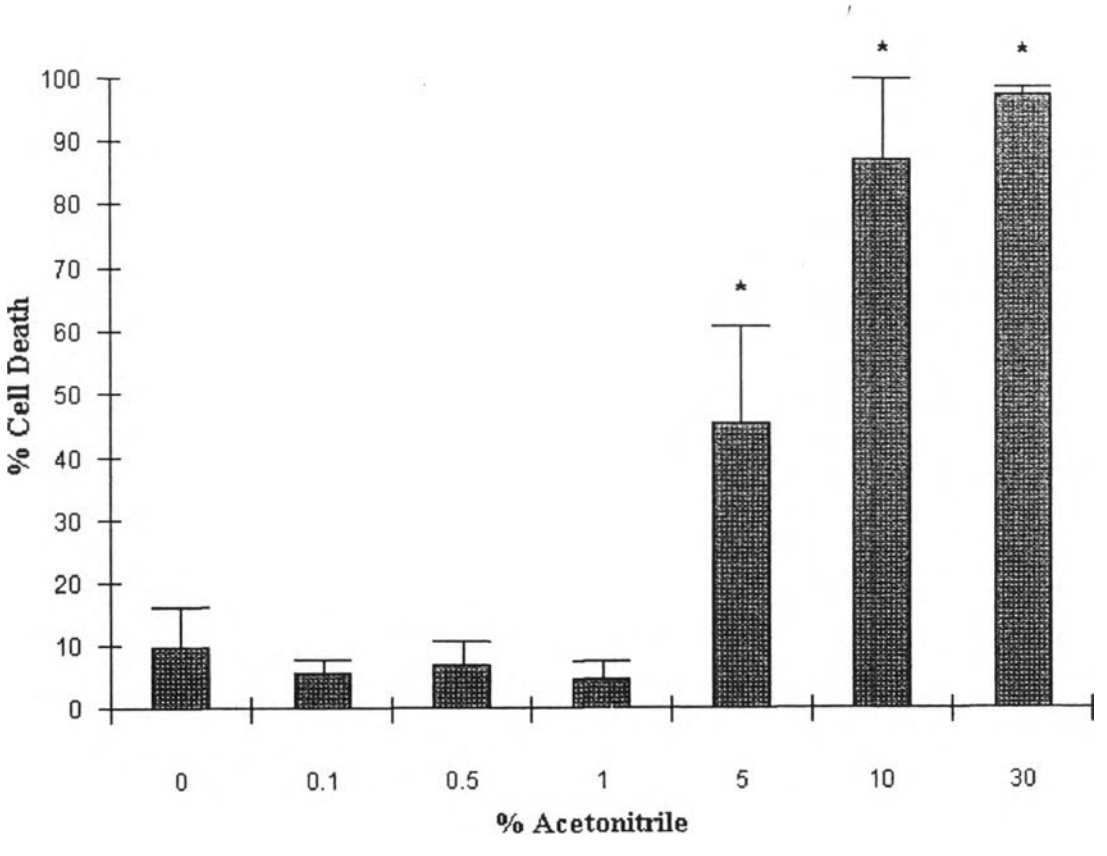

Figure 38 Graph shows percentage of Jurkat cell death detected by annexin V-FITC after acetonitrile exposure for 6 hours. (* $p<0.05$)

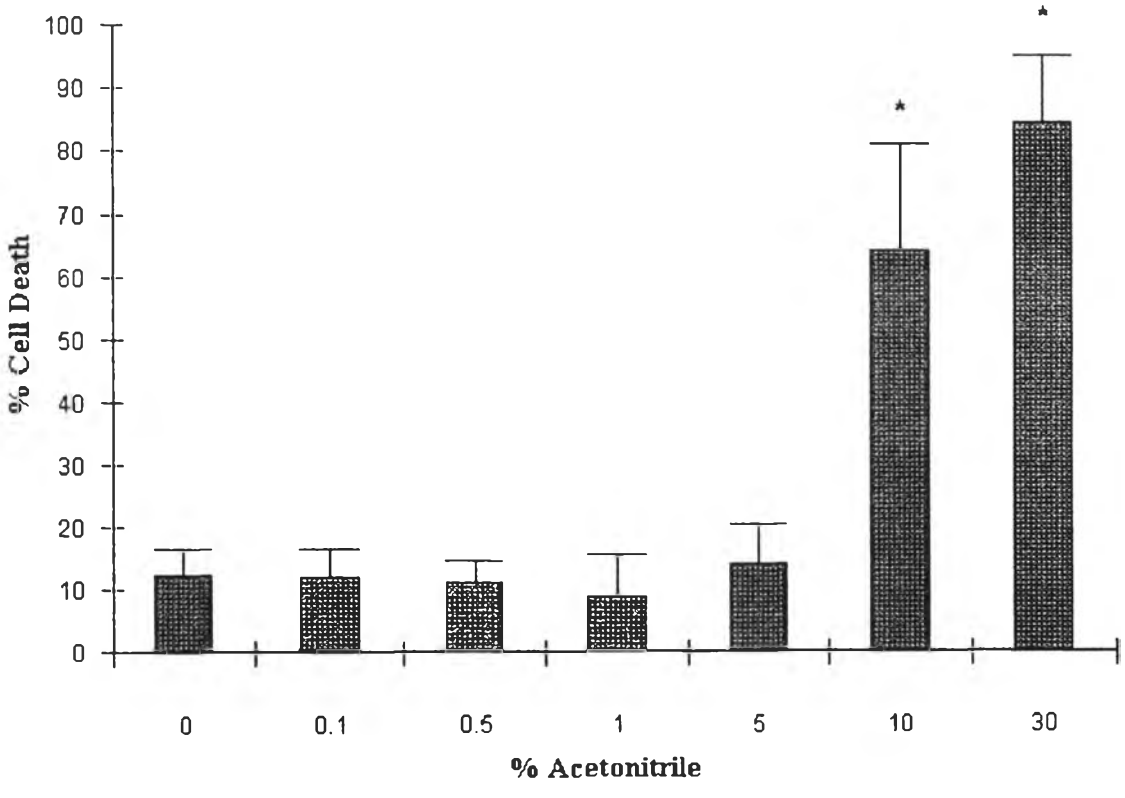

Figure 39 Graph shows percentage of Raji cell death detected by annexin V-FITC after acetonitrile exposure for 6 hours. (* $p<0.05$)

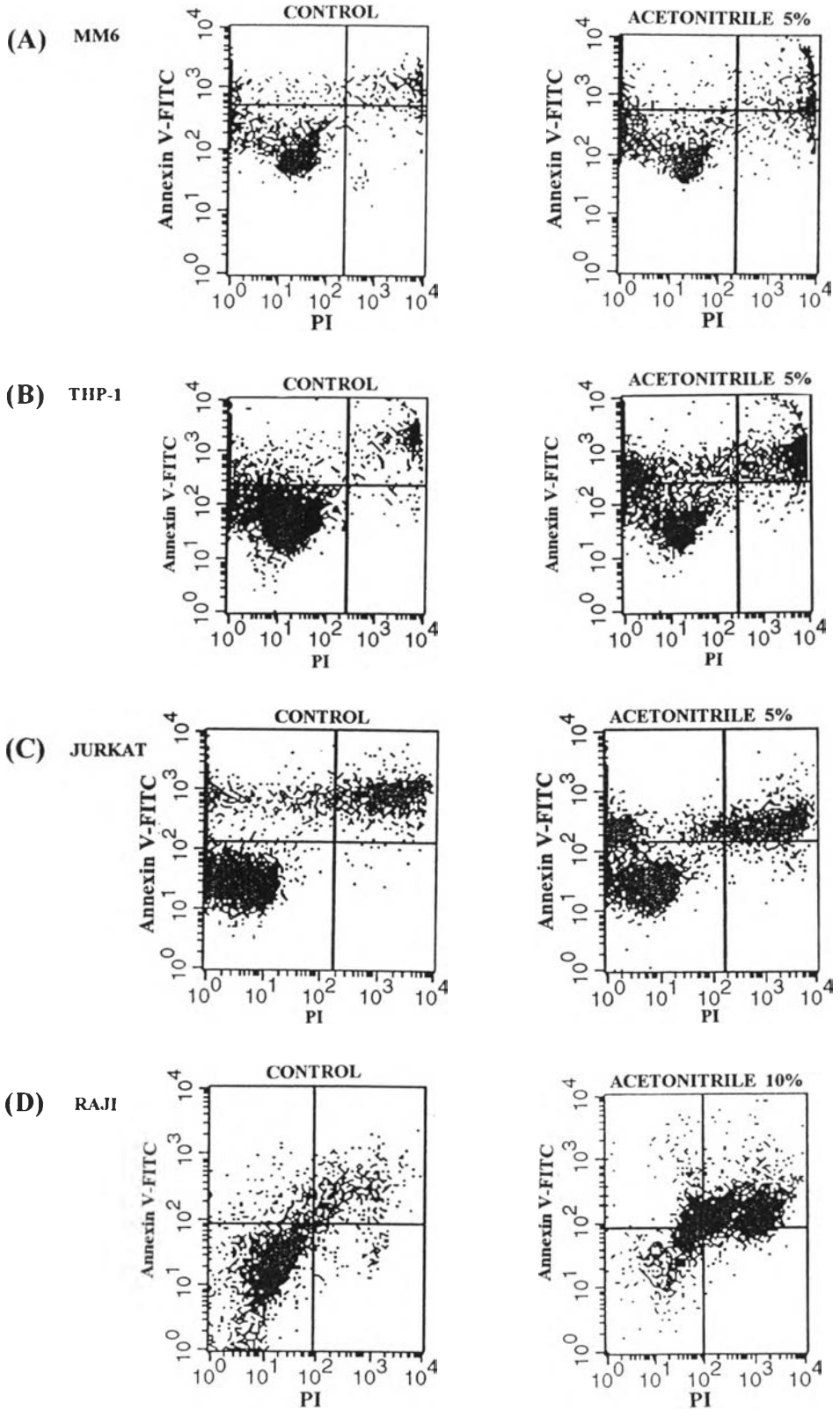

Figure 40 Flow cytometric analysis of cell death detected by annexin V-FITC after acetonitrile exposure for 6 hours. (A) MM6, (B) THP-1, (C) Jurkat, and (D) Raji.

The Reduction of Mitrochondrial Transmembrane Potential in MM6, THP-1, Jurkat, and Raji Cells Detected by TMRE

Acetonitrile Exposure for 6 Hours:

Table 32 Reduction of mitochondrial transmembrane potential in MM6 cells detected by TMRE after acetonitrile exposure for 6 hours. (* $p<0.05$, compared to control)

% Acetonitrile	% Cells with $\downarrow\Delta\Psi$ (Mean ± S.E.)
0 (control)	13.8 ± 6.438
0.1	13.89 ± 6.783
0.5	9.68 ± 7.19
1	10.32 ± 8.28
5	76.07 ± 19.01*
10	91.99 ± 15.21*
30	99.96 ± 0.05*

Table 33 Reduction of mitochondrial transmembrane potential in THP-1 cells detected by TMRE after acetonitrile exposure for 6 hours. (* $p<0.05$, compared to control)

% Acetonitrile	% Cells with $\downarrow\Delta\Psi$ (Mean ± S.E.)
0 (control)	14.25 ± 3.515
0.1	13.83 ± 3.182
0.5	10.82 ± 2.364
1	12.27 ± 5.39
5	42.31 ± 19.18*
10	85.61 ± 16.15*
30	99.10 ± 1.327*

Table 34 Reduction of mitochondrial transmembrane potential in Jurkat cells detected by TMRE after acetonitrile exposure for 6 hours. (* p<0.05, compared to control)

% Acetonitrile	% Cells with $\downarrow\Delta\Psi$ (Mean ± S.E.)
0 (control)	9.69 ± 1.32
0.1	11.24 ± 2.347
0.5	14.55 ± 7.628
1	11.76 ± 4.603
5	65.41 ± 24.31*
10	99.69 ± 0.559*
30	99.25 ± 1.283*

Table 35 Reduction of mitochondrial transmembrane potential in Raji cells detected by TMRE after acetonitrile exposure for 6 hours. (* p<0.05, compared to control)

% Acetonitrile	% Cells with $\downarrow\Delta\Psi$ (Mean ± S.E.)
0 (control)	9.75 ± 5.808
0.1	9.64 ± 4.458
0.5	11.95 ± 5.614
1	11.64 ± 4.335
5	17.94 ± 10.72
10	99.63 ± 0.739*
30	99.84 ± 0.613*

Acetonitrile Exposure for 6 Hours:

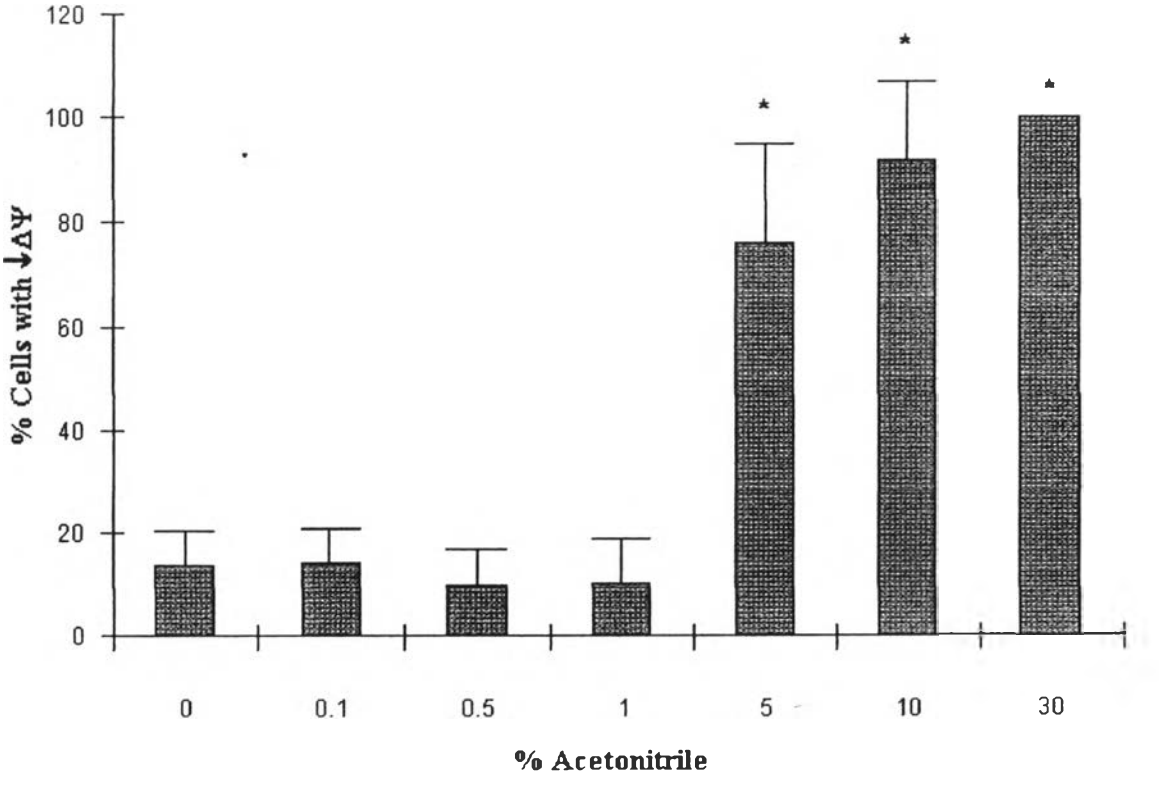

Figure 41 Graph shows percentage of MM6 cells with the reduction of mitochondrial transmembrane potential detected by TMRE after acetonitrile exposure for 6 hours. (* $p<0.05$)

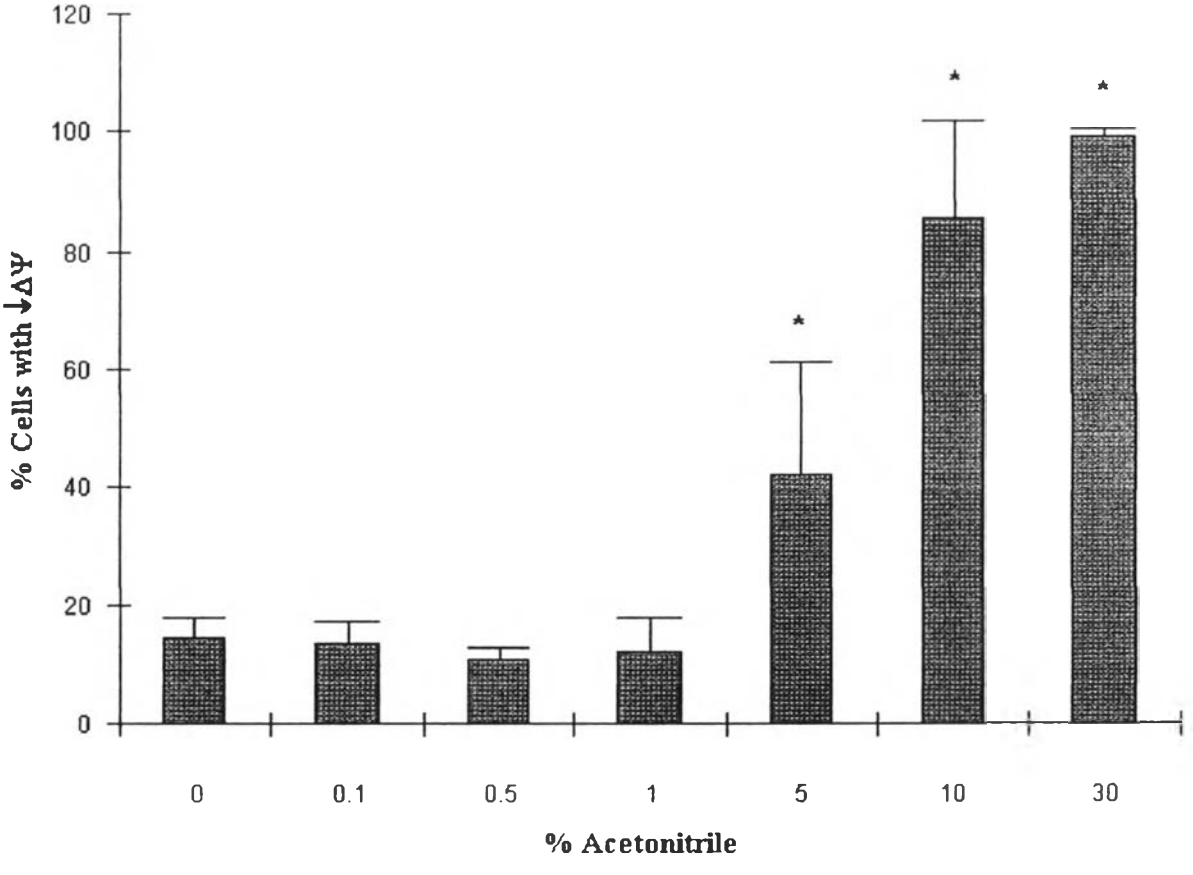

Figure 42 Graph shows percentage of THP-1 cells with the reduction of mitochondrial transmembrane potential detected by TMRE after acetonitrile exposure for 6 hours. (* $p<0.05$)

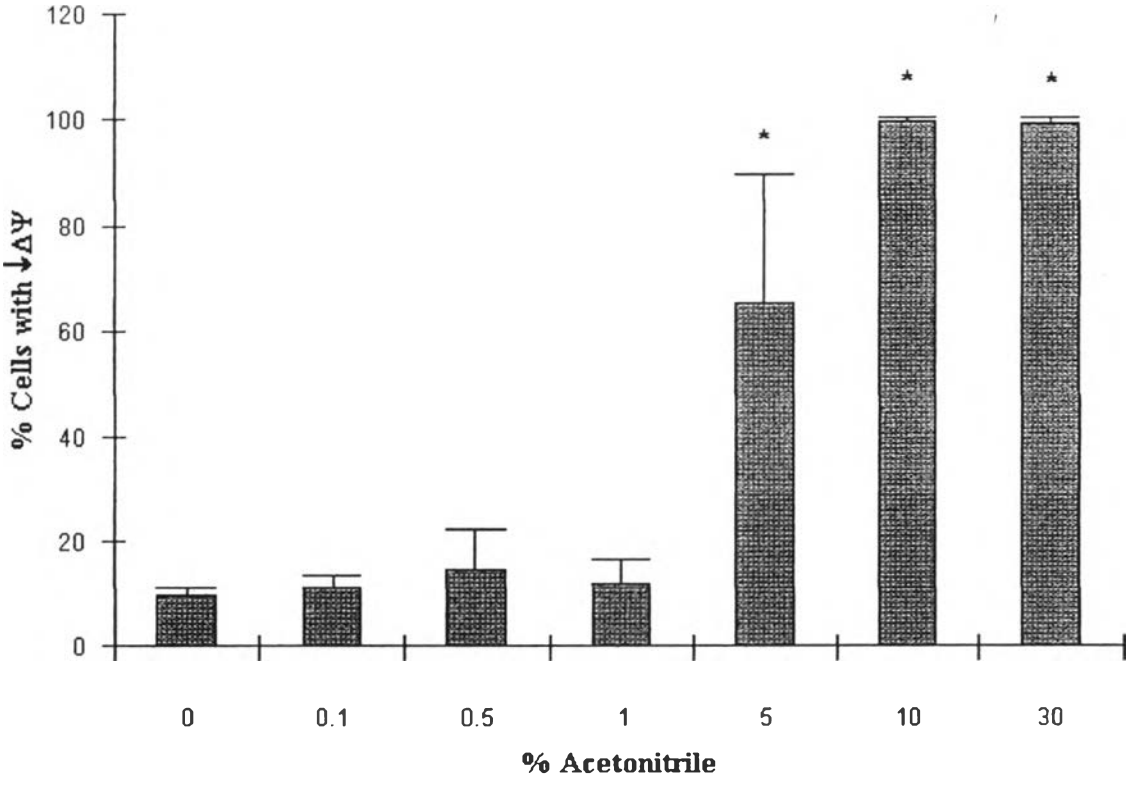

Figure 43 Graph shows percentage of Jurkat cells with the reduction of mitochondrial transmembrane potential detected by TMRE after acetonitrile exposure for 6 hours. (* $p<0.05$)

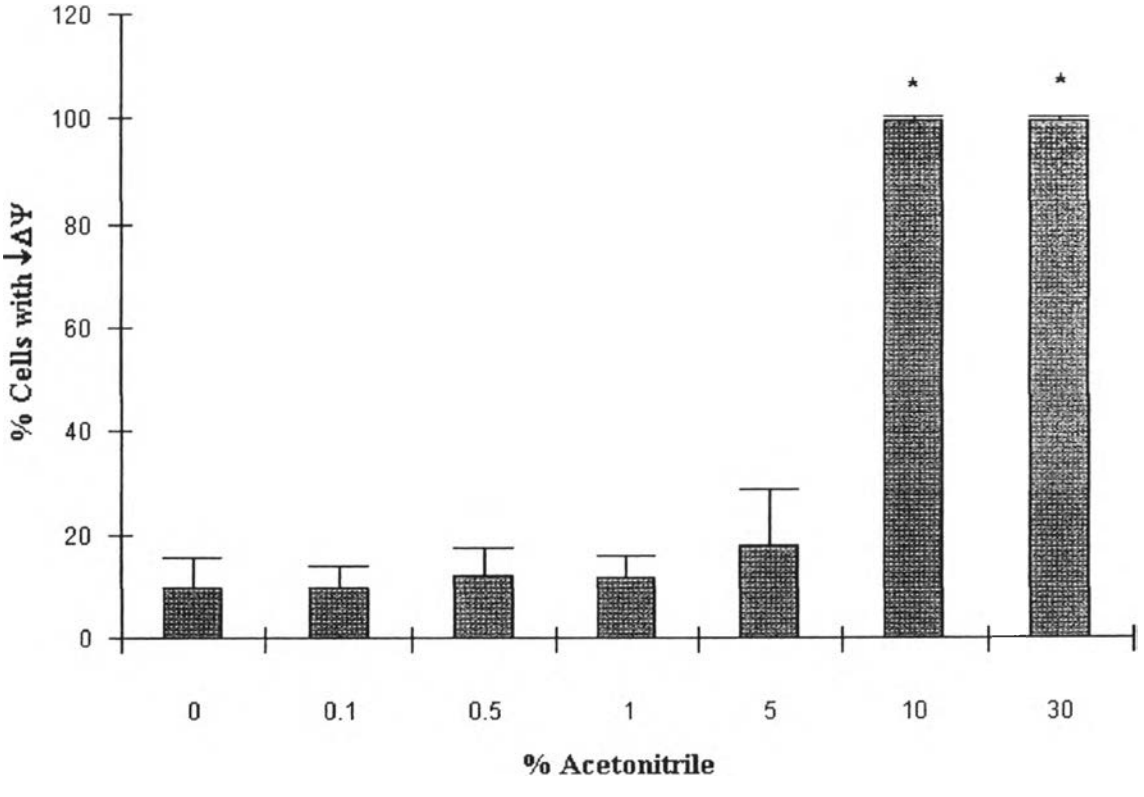

Figure 44 Graph shows percentage of Raji cells with the reduction of mitochondrial transmembrane potential detected by TMRE after acetonitrile exposure for 6 hours. (* $p<0.05$)

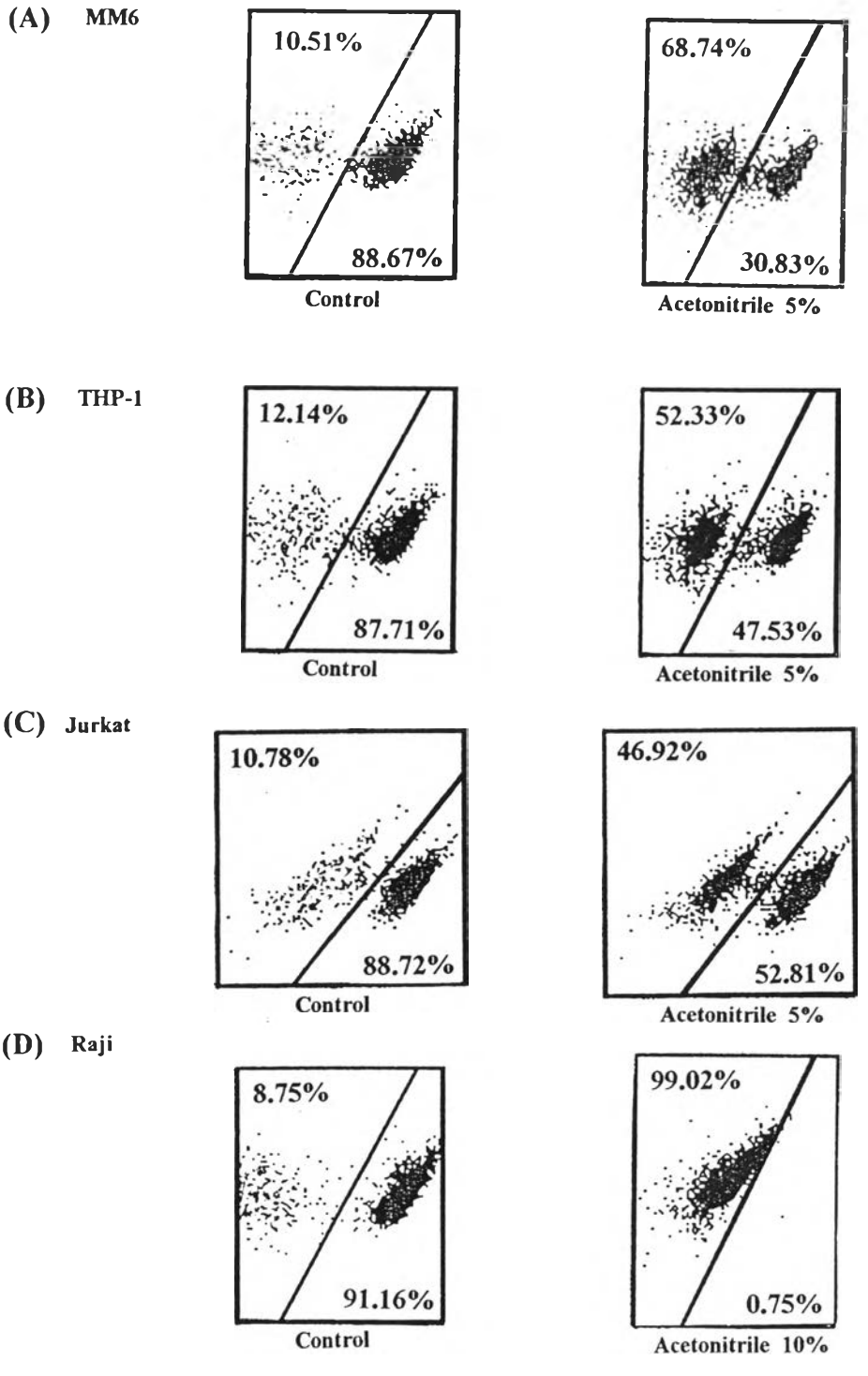

Figure 45 Flow cytometric analysis of the reduction of mitochondrial transmembrane potential detected by TMRE after acetonitrile exposure for 6 hours. (A) MM6, (B), THP-1, (C) Jurkat, and (D) Raji. Cells with the lower red fluorescence (TMRE) (or cells in the left gate) are cells with $\downarrow\Delta\Psi$.

DNA Fragmentation of in MM6, THP-1, Jurkat, and Raji Cells Shown in the Sub-G_1 Area Detected by PI

Acetonitrile Exposure for 6 Hours:

Table 36 Increase of MM6 cells in the sub-G_1 area detected by PI after acetonitrile exposure for 6 hours. (* $p<0.05$, compared to control)

% Acetonitrile	% Cells in the Sub-G_1 Area (Mean ± S.E.)
0 (control)	9.42 ± 3.347
0.1	9.25 ± 3.854
0.5	8.39 ± 3.751
1	7.93 ± 3.958
5	21.75 ± 5.321*
10	22.80 ± 4.043*
30	26.57 ± 3.769*

Table 37 Increase of THP-1 cells in the sub-G_1 area detected by PI after acetonitrile exposure for 6 hours. (* $p<0.05$, compared to control)

% Acetonitrile	% Cells in the Sub-G_1 Area (Mean ± S.E.)
0 (control)	7.79 ± 3.98
0.1	7.68 ± 4.147
0.5	7.45 ± 3.763
1	11.64 ± 4.335
5	17.54 ± 3.98*
10	21.75 ± 4.183*
30	19.97 ± 4.295*

Table 38 Increase of Jurkat cells in the sub-G_1 area detected by PI after acetonitrile exposure for 6 hours. (* $p<0.05$, compared to control)

% Acetonitrile	% Cells in the Sub-G_1 Area (Mean ± S.E.)
0 (control)	6.06 ± 3.128
0.1	3.79 ± 4.73
0.5	4.25 ± 4.871
1	4.43 ± 4.752
5	19.37 ± 6.284*
10	21.17 ± 4.906*
30	23.86 ± 5.178*

Table 39 Increase of Raji cells in the sub-G_1 area detected by PI after acetonitrile exposure for 6 hours. (* $p<0.05$, compared to control)

% Acetonitrile	% Cells in the Sub-G_1 Area (Mean ± S.E.)
0 (control)	9.45 ± 3.402
0.1	9.81 ± 3.89
0.5	10.45 ± 4.647
1	8.96 ± 4.18
5	14.67 ± 4.257
10	21.81 ± 3.988*
30	23.59 ± 4.516*

Acetonitrile Exposure for 6 Hours:

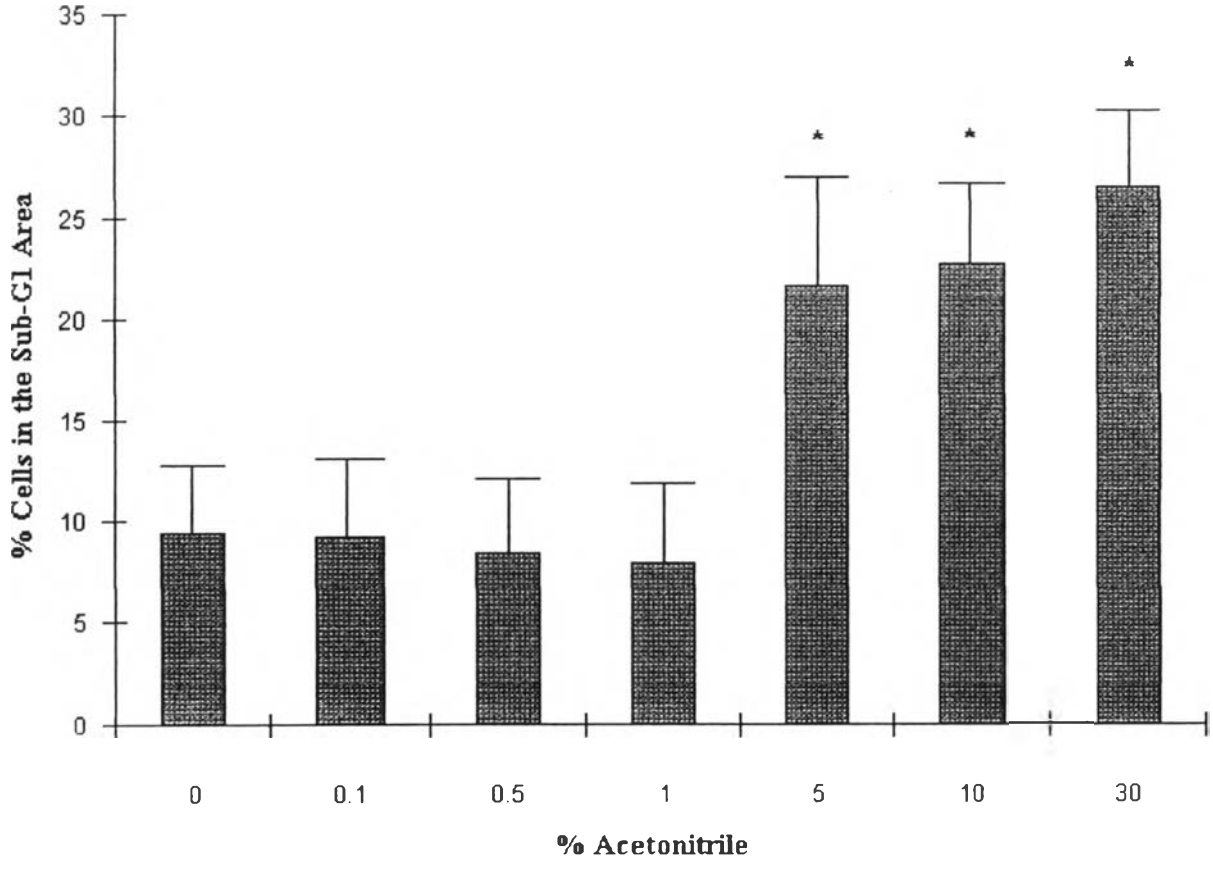

Figure 46 Graph shows percentage of MM6 cells in the sub-G_1 area detected by PI after acetonitrile exposure for 6 hours. (* $p<0.05$)

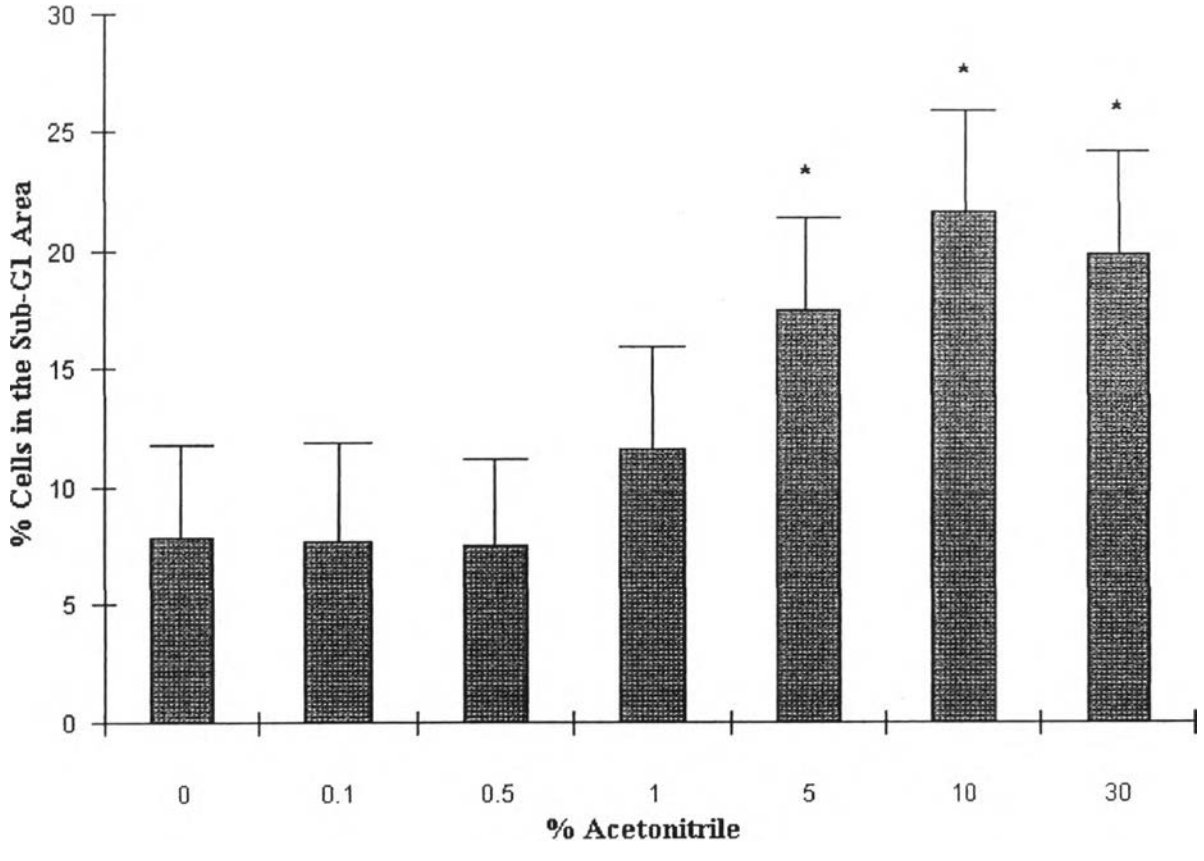

Figure 47 Graph shows percentage of THP-1 cells in the sub-G_1 area detected by PI after acetonitrile exposure for 6 hours. (* $p<0.05$)

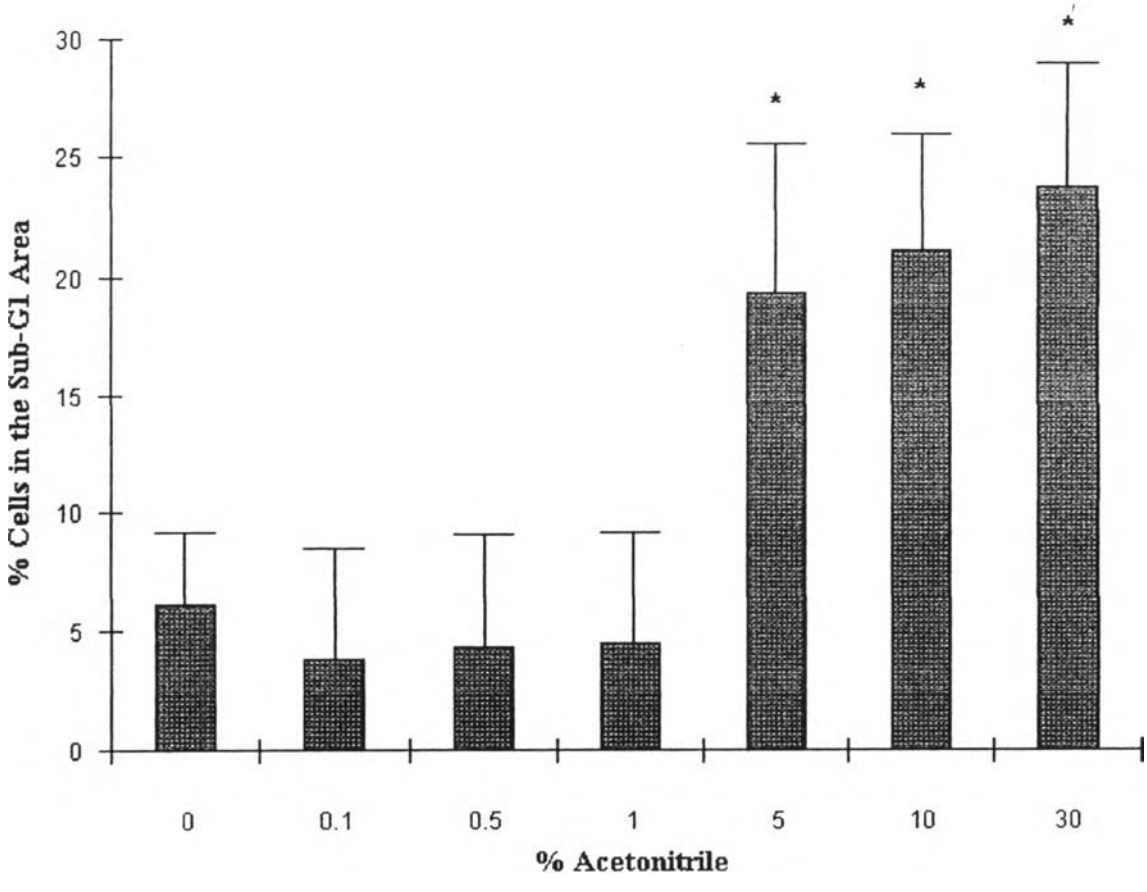

Figure 48 Graph shows percentage of Jurkat cells in the sub-G_1 area detected by PI after acetonitrile exposure for 6 hours. (* $p<0.05$)

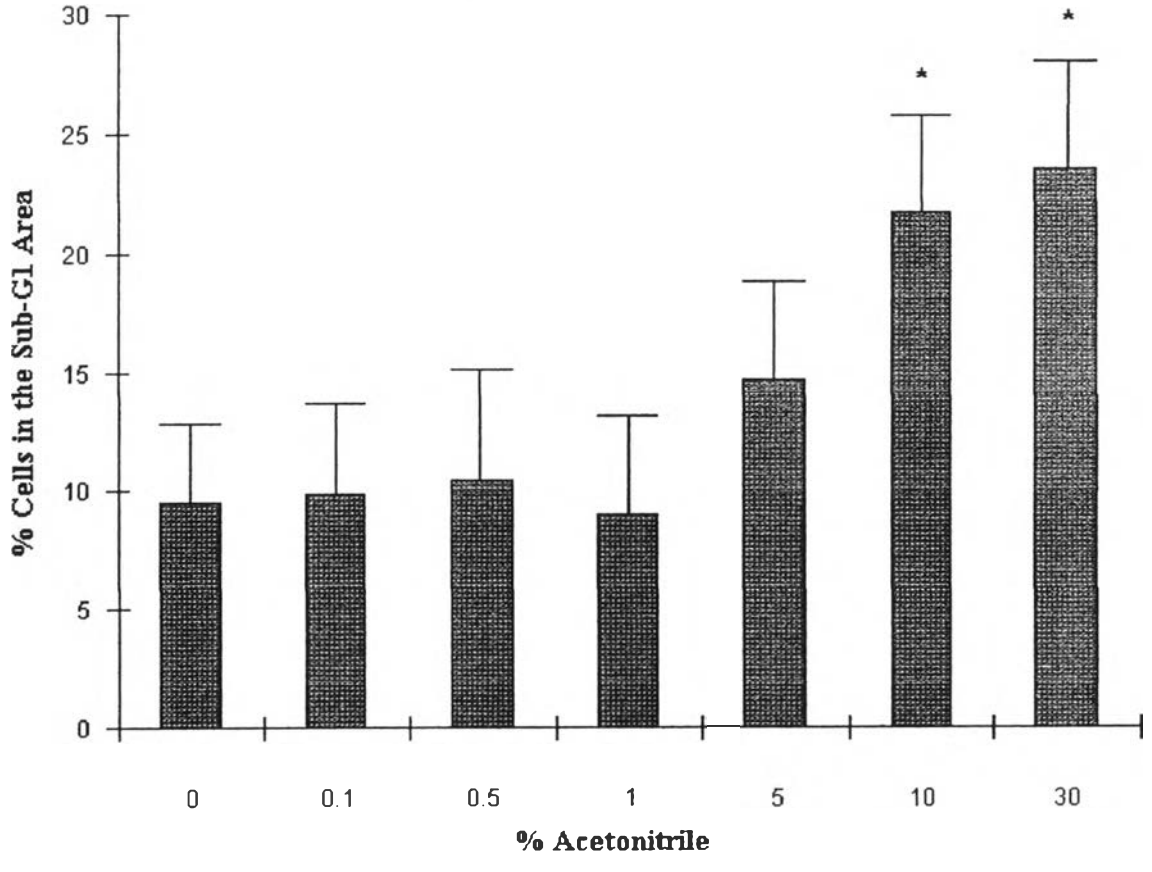

Figure 49 Graph shows percentage of Raji cells in the sub-G_1 area detected by PI after acetonitrile exposure for 6 hours. (* $p<0.05$)

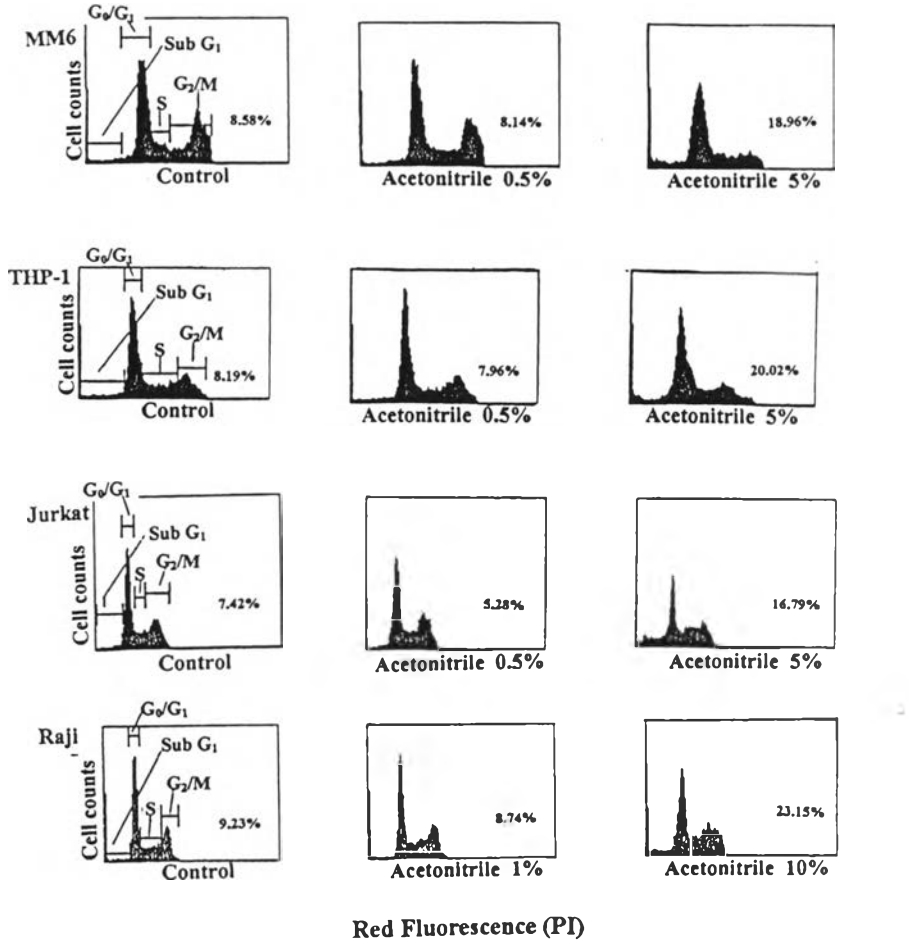

Figure 50 Flow cytometric analysis of cells with DNA fragmentation in the sub-G_1 area detected by PI after acetonitrile exposure for 6 hours. (A) MM6, (B), THP-1, (C) Jurkat, and (D) Raji. The increases in percentage of cells in the sub-G_1 area are shown.

Role of Caspase on Methomyl-Induced Cell Death

Exposure of THP-1 to zVAD-fmk and Methomyl for 6 Hours:

Table 40 Effects of 1 μM zVAD-fmk on THP-1 cell death detected by annexin V-FITC after methomyl exposure for 6 hours.

Treatments	% THP-1 cell death (Mean ± S.E.)
Methomyl 0 mM (Control)	11.7 ± 5.214
Control + zVAD-fmk (z) 1 μM	8.35 ± 3.465
Methomyl (M) 12 mM	26.17 ± 6.891
M 12 mM + z 1 μM	20.78 ± 6.304
M 24 mM	38.23 ± 4.973
M 24 mM + z 1 μM	37.34 ± 6.18
M 30 mM	50.52 ± 6.933
M 30 mM + z 1 μM	53.71 ± 6.633

Table 41 Effects of 10 μM zVAD-fmk on THP-1 cell death detected by annexin V-FITC after methomyl exposure for 6 hours. (* $p<0.05$, comparison between zVAD-fmk-treated- and -untreated-group at the same dose of methomyl)

Treatments	% THP-1 cell death (Mean ± S.E.)
Methomyl 0 mM (Control)	11.7 ± 5.214
Control + zVAD-fmk (z) 10 μM	6.2 ± 5.403
Methomyl (M) 12 mM	26.17 ± 6.891
M 12 mM + z 10 μM	14.01 ± 5.834
M 24 mM	38.23 ± 4.973
M 24 mM + z 10 μM	23.02 ± 4.875*
M 30 mM	50.52 ± 6.933
M 30 mM + z 10 μM	17.91 ± 5.89*

Table 42 Effects of 100 μM zVAD-fmk on THP-1 cell death detected by annexin V-FITC after methomyl exposure for 6 hours. (* $p<0.05$, comparison between zVAD-fmk-treated- and -untreated-group at the same dose of methomyl)

Treatments	% THP-1 cell death (Mean ± S.E.)
Methomyl 0 mM (Control)	11.7 ± 5.214
Control + zVAD-fmk (z) 100 μM	6.65 ± 3.132
Methomyl (M) 12 mM	26.17 ± 6.891
M 12 mM + z 100 μM	7.11 ± 3.56*
M 24 mM	38.23 ± 4.973
M 24 mM + z 100 μM	5.27 ± 3.808*
M 30 mM	50.52 ± 6.933
M 30 mM + z 100 μM	7.78 ± 2.967*

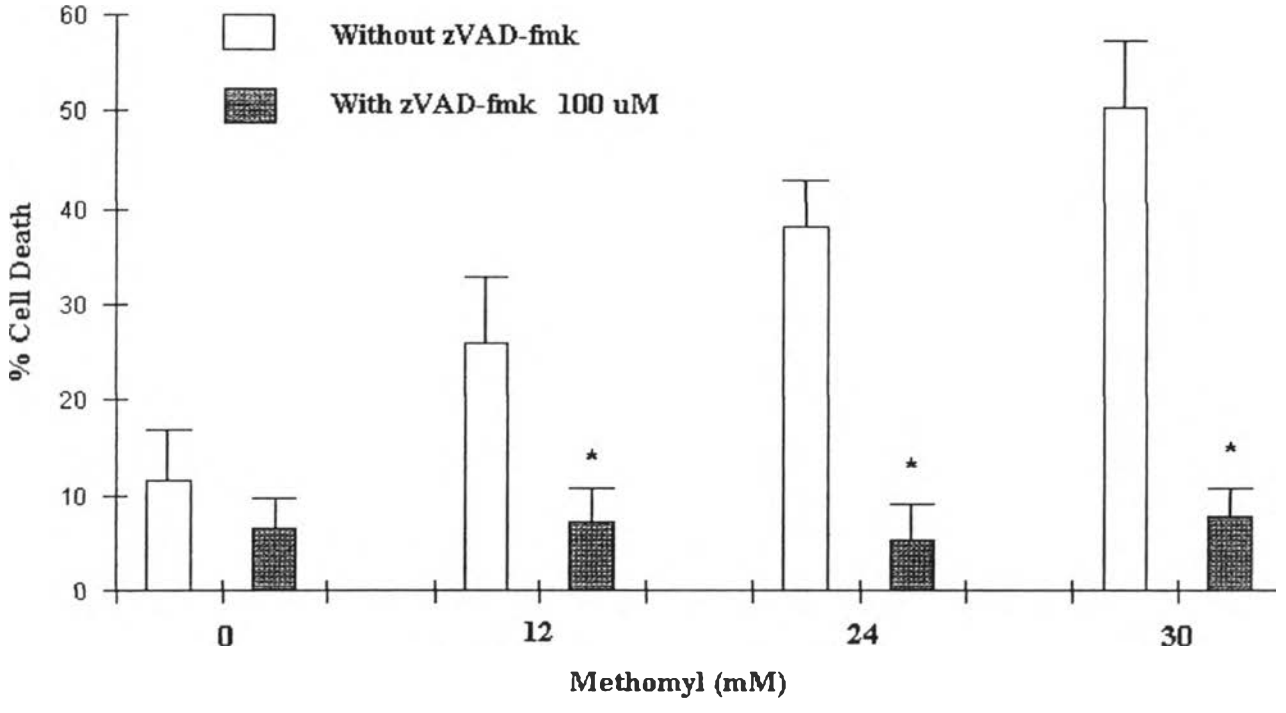

Figure 51 Graph shows effects of 100 μM zVAD-fmk on THP-1 cell death detected by annexin V-FITC after methomyl exposure for 6 hours. (* $p<0.05$, comparison between zVAD-fmk-treated- and -untreated-group at the same dose of methomyl)

Table 43 Effects of 1 μM zVAD-fmk on the reduction of mitochondrial transmembrane potential in THP-1 cells detected by TMRE after methomyl exposure for 6 hours.

Treatments	% Cells with ↓ΔΨ (Mean ± S.E.)
Methomyl 0 mM (Control)	13.47 ± 5.463
Control + zVAD-fmk (z) 1 μM	15.83 ± 3.641
Methomyl (M) 12 mM	47.53 ± 7.811
M 12 mM + z 1 μM	41.43 ± 5.617
M 24 mM	73.58 ± 7.654
M 24 mM + z 1 μM	67.66 ± 4.159
M 30 mM	86.89 ± 5.232
M 30 mM + z 1 μM	88.36 ± 5.135

Table 44 Effects of 10 μM zVAD-fmk on the reduction of mitochondrial transmembrane potential in THP-1 cells detected by TMRE after methomyl exposure for 6 hours. (* $p<0.05$, comparison between zVAD-fmk-treated- and -untreated-group at the same dose of methomyl)

Treatments	% Cells with ↓ΔΨ (Mean ± S.E.)
Methomyl 0 mM (Control)	13.47 ± 5.463
Control + zVAD-fmk (z) 10 μM	12.43 ± 3.171
Methomyl (M) 12 mM	47.53 ± 7.811
M 12 mM + z 10 μM	28.91 ± 5.485*
M 24 mM	73.58 ± 7.654
M 24 mM + z 10 μM	46.01 ± 3.788*
M 30 mM	86.89 ± 5.232
M 30 mM + z 10 μM	42.9 ± 3.753*

Table 45 Effects of 100 μM zVAD-fmk on the reduction of mitochondrial transmembrane potential in THP-1 cells detected by TMRE after methomyl exposure for 6 hours. (* $p<0.05$, comparison between zVAD-fmk-treated- and -untreated-group at the same dose of methomyl)

Treatments	% THP-1 cell death (Mean ± S.E.)
Methomyl 0 mM (Control)	13.47 ± 5.463
Control + zVAD-fmk (z) 100 μM	7.66 ± 2.916
Methomyl (M) 12 mM	47.53 ± 7.811
M 12 mM + z 100 μM	16.61 ± 4.119 *
M 24 mM	73.58 ± 7.654
M 24 mM + z 100 μM	19.57 ± 3.11 *
M 30 mM	86.89 ± 5.232
M 30 mM + z 100 μM	38.39 ± 3.809 *

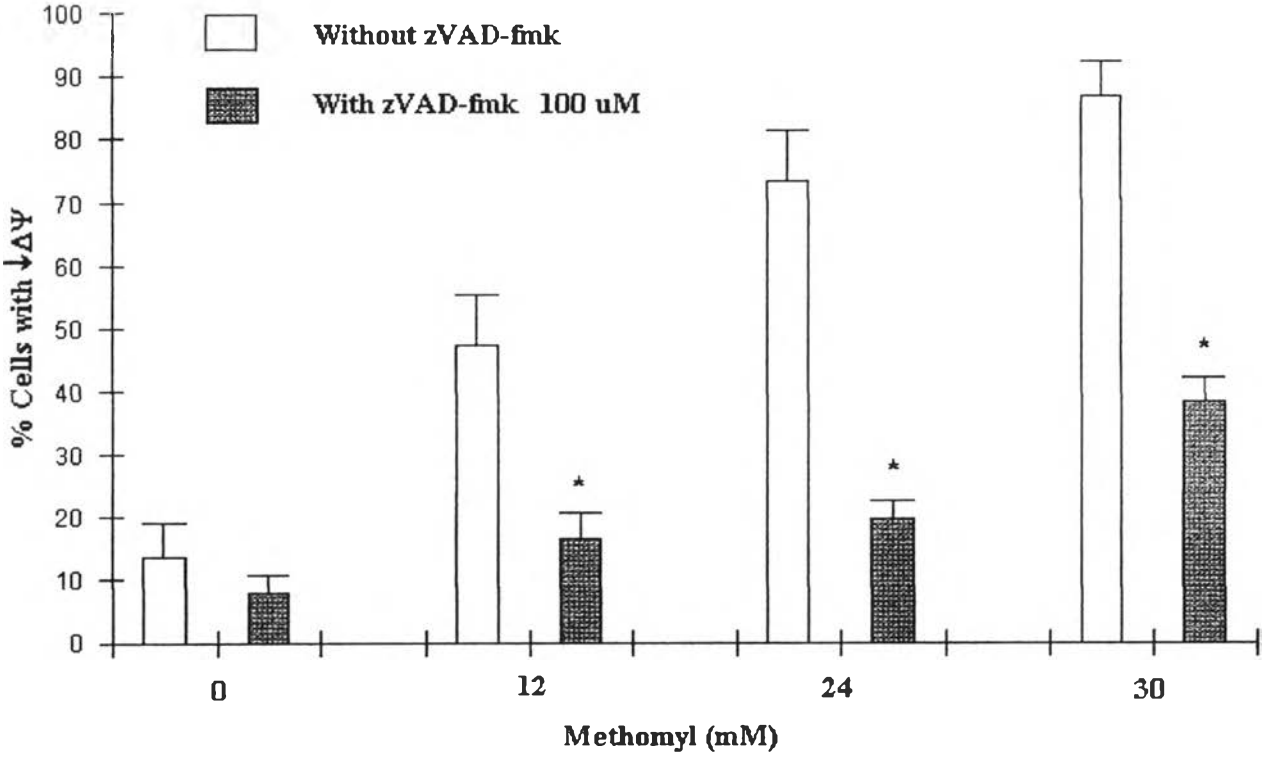

Figure 52 Graph shows effects of 100 μM zVAD-fmk on the reduction of mitochondrial transmembrane potential in THP-1 cells detected by TMRE after methomyl exposure for 6 hours. (* $p<0.05$, comparison between zVAD-fmk-treated- and -untreated-group at the same dose of methomyl)

Role of Caspase-3 on Methomyl- and Acetonitrile-Induced Cell Death Detected by Immunoblot

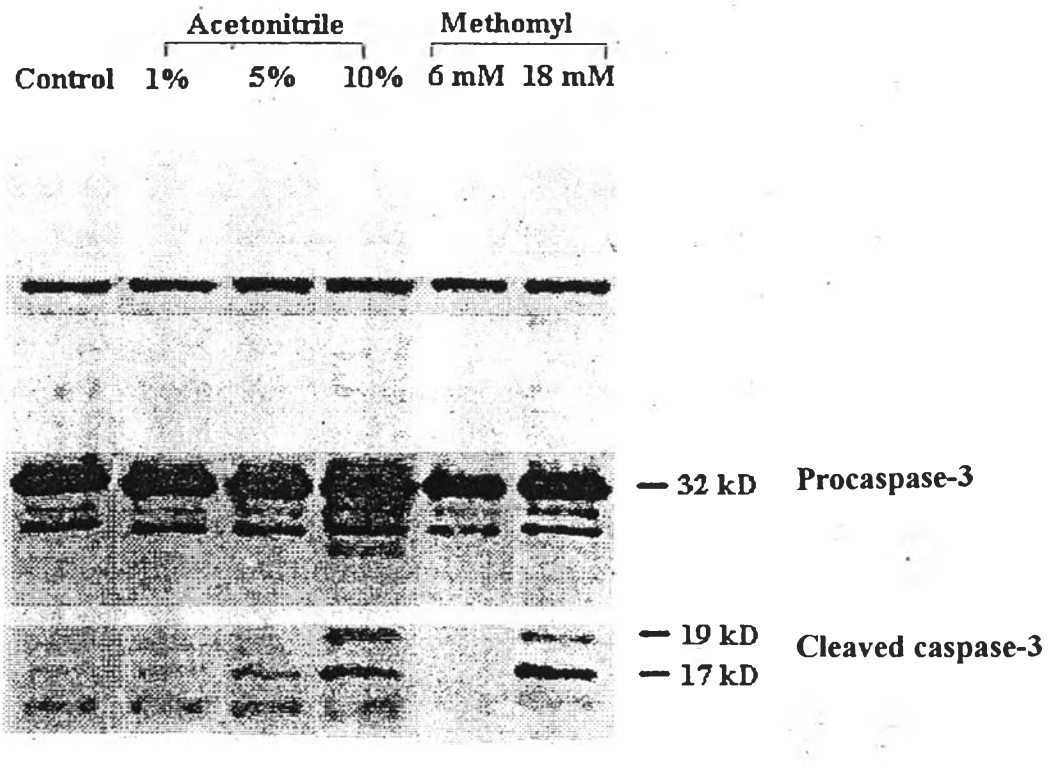

Figure 53 Immunoblot analysis of caspase-3 cleavage in Jurkat cells after methomyl and acetonitrile exposure for 6 hours.

Effect of IL-6 on Methomyl-Induced Cell Death

Exposure to IL-6 and Methomyl for 6 Hours:

Table 46 Effects of 50 nM IL-6 on the reduction of mitochondrial transmembrane potential in MM6 cells detected by TMRE after methomyl exposure for 6 hours. (* $p<0.05$, comparison between IL-6-treated- and -untreated-group at the same dose of methomyl)

Treatments	% Cells with $\downarrow\Delta\Psi$ (Mean ± S.E.)
Methomyl 0 mM (Control)	12.48 ± 3.828
Control + IL-6 50 ng/ml	10.94 ± 2.46
Methomyl (M) 6 mM	11.6 ± 3.517
M 6 mM + IL-6 50 ng/ml	10.18 ± 1.785
M 12 mM	11.23 ± 3.659
M 12 mM + IL-6 50 ng/ml	10.68 ± 4.235
M 18 mM	48.04 ± 7.061
M 18 mM + IL-6 50 ng/ml	47.07 ± 13.18
M 24 mM	84.03 ± 2.035
M 24 mM + IL-6 50 ng/ml	77.19 ± 3.925 *
M 30 mM	96.33 ± 1.233
M 30 mM + IL-6 50 ng/ml	89.45 ± 3.029 *

Table 47 Effects of 50 nM IL-6 on the reduction of mitochondrial transmembrane potential in THP-1 cells detected by TMRE after methomyl exposure for 6 hours. (* $p<0.05$, comparison between IL-6-treated- and -untreated-group at the same dose of methomyl)

Treatments	% Cells with $\downarrow\Delta\Psi$ (Mean ± S.E.)
Methomyl 0 mM (Control)	14.65 ± 5.82
Control + IL-6 50 ng/ml	9.6 ± 1.039
Methomyl (M) 6 mM	15.23 ± 6.234
M 6 mM + IL-6 50 ng/ml	9.433 ± 3.128
M 12 mM	27.28 ± 5.562
M 12 mM + IL-6 50 ng/ml	25.68 ± 6.087
M 18 mM	44.36 ± 4.401
M 18 mM + IL-6 50 ng/ml	32.26 ± 4.907
M 24 mM	74.34 ± 10.33
M 24 mM + IL-6 50 ng/ml	66.45 ± 7.092
M 30 mM	97.61 ± 9.576
M 30 mM + IL-6 50 ng/ml	79.14 ± 8.761 *

Table 48 Effects of 50 nM IL-6 on the reduction of mitochondrial transmembrane potential in Jurkat cells detected by TMRE after methomyl exposure for 6 hours. (* $p<0.05$, comparison between IL-6-treated- and -untreated-group at the same dose of methomyl)

Treatments	% Cells with $\downarrow\Delta\Psi$ (Mean ± S.E.)
Methomyl 0 mM (Control)	14.08 ± 3.554
Control + IL-6 50 ng/ml	14.64 ± 3.319
Methomyl (M) 6 mM	18.01 ± 4.185
M 6 mM + IL-6 50 ng/ml	15.56 ± 2.869
M 12 mM	31.23 ± 3.659
M 12 mM + IL-6 50 ng/ml	28.68 ± 4.235
M 18 mM	44.36 ± 4.401
M 18 mM + IL-6 50 ng/ml	32.26 ± 4.907*
M 24 mM	72.58 ± 3.104
M 24 mM + IL-6 50 ng/ml	64.06 ± 3.746*
M 30 mM	94.03 ± 2.134
M 30 mM + IL-6 50 ng/ml	77.61 ± 4.710 *

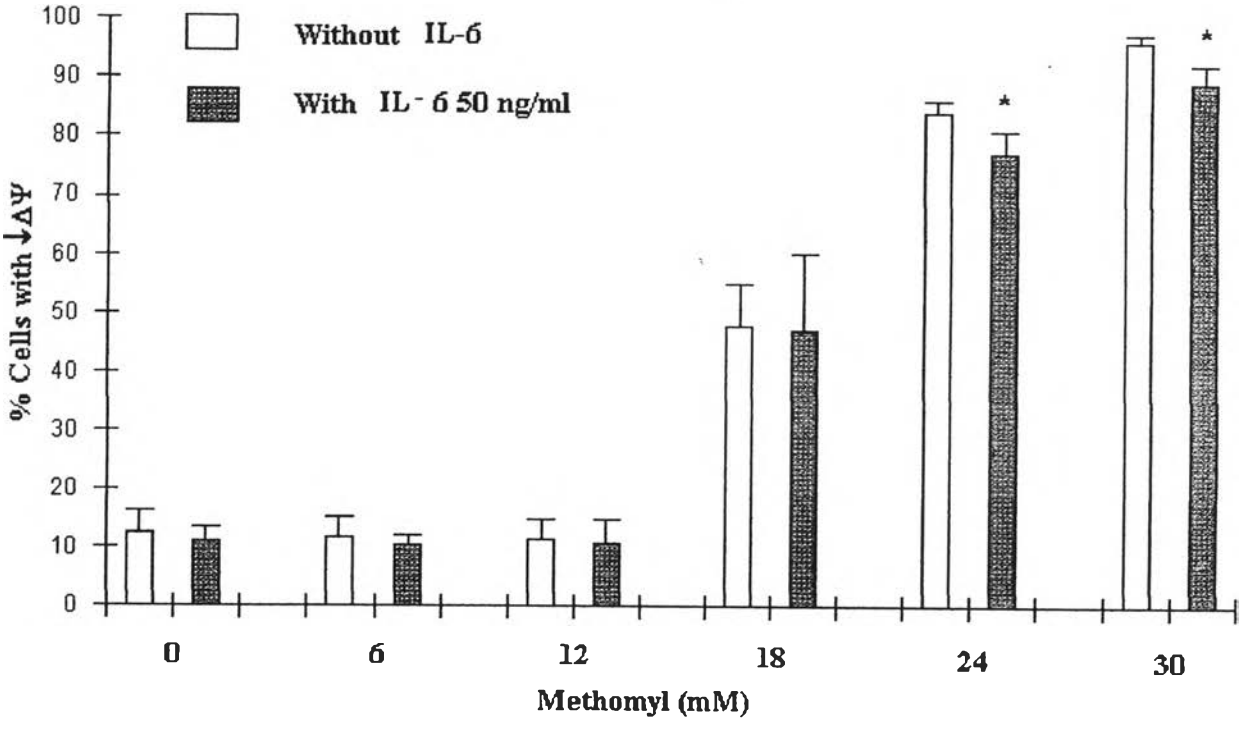

Figure 54 Graph shows effects of 50 nM IL-6 on the reduction of mitochondrial transmembrane potential in MM6 cells detected by TMRE after methomyl exposure for 6 hours. (* $p<0.05$, comparison between IL-6-treated- and -untreated-group at the same dose of methomyl)

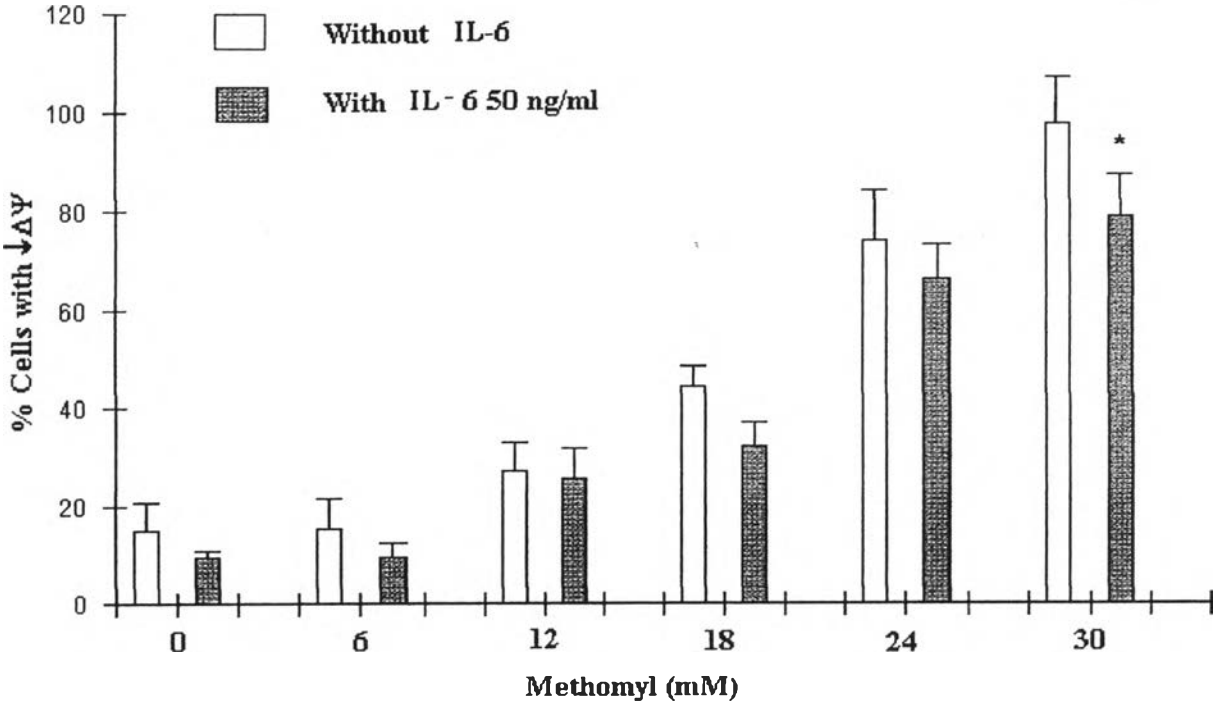

Figure 55 Graph shows effects of 50 nM IL-6 on the reduction of mitochondrial transmembrane potential in THP-1 cells detected by TMRE after methomyl exposure for 6 hours. (* $p<0.05$, comparison between IL-6-treated- and -untreated-group at the same dose of methomyl)

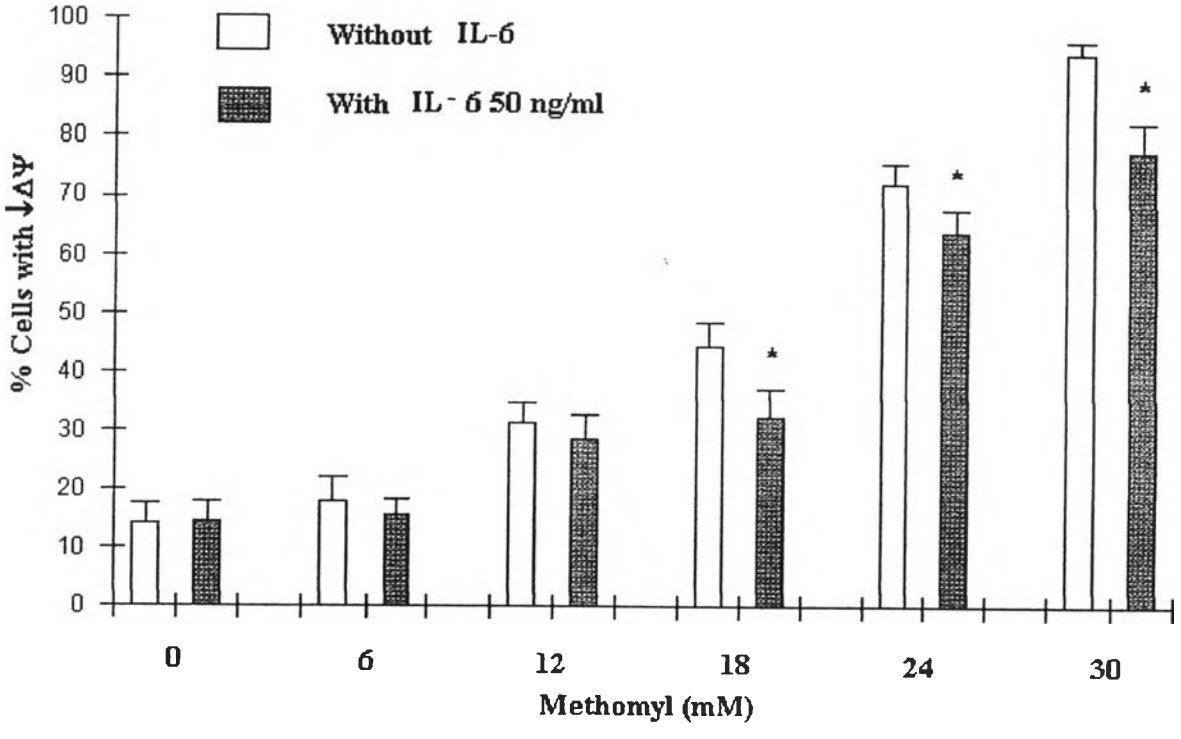

Figure 56 Graph shows effects of 50 nM IL-6 on the reduction of mitochondrial transmembrane potential in Jurkat cells detected by TMRE after methomyl exposure for 6 hours. (* $p<0.05$, comparison between IL-6-treated- and -untreated-group at the same dose of methomyl)

In Vivo-Results

Single dose of methomyl oral treatment at 8 mg/kg body weight could induce apoptosis in lymphocytes after 6 hours of the exposure.

Transmission electron microscopic sections of spleens collected from rats after the 24 hour-oral exposure to single dose of methomyl at 8 mg/kg illustrated the mitochondrial swelling and degenerative changes with cristae loss. Cell death was shown under light microscope at the same dose of methomyl (data not shown).

In addition, energy metabolism related to mitochondrial function was investigated. The results showed that the increasing levels of 2,3-DPG in blood and the increase in red blood cell NADH-DCIP reductase activity were affected in dose-response relationship.

Effect of Methomyl on Lymphocytes

Table 49 Rat lymphocytic cell death detected by annexin V-FITC after single dose oral-treatments of methomyl for 6 hours. (* $p<0.05$, compared to control)

Methomyl Treatment (mg/kg body weight) (n=6)	% Cell Death (Mean ± S.E.)
Methomyl 0 mg/kg (Control)	1.877 ± 1.262
Methomyl 4 mg/kg	1.685 ± 1.888
Methomyl 8 mg/kg	4.905 ± 1.674

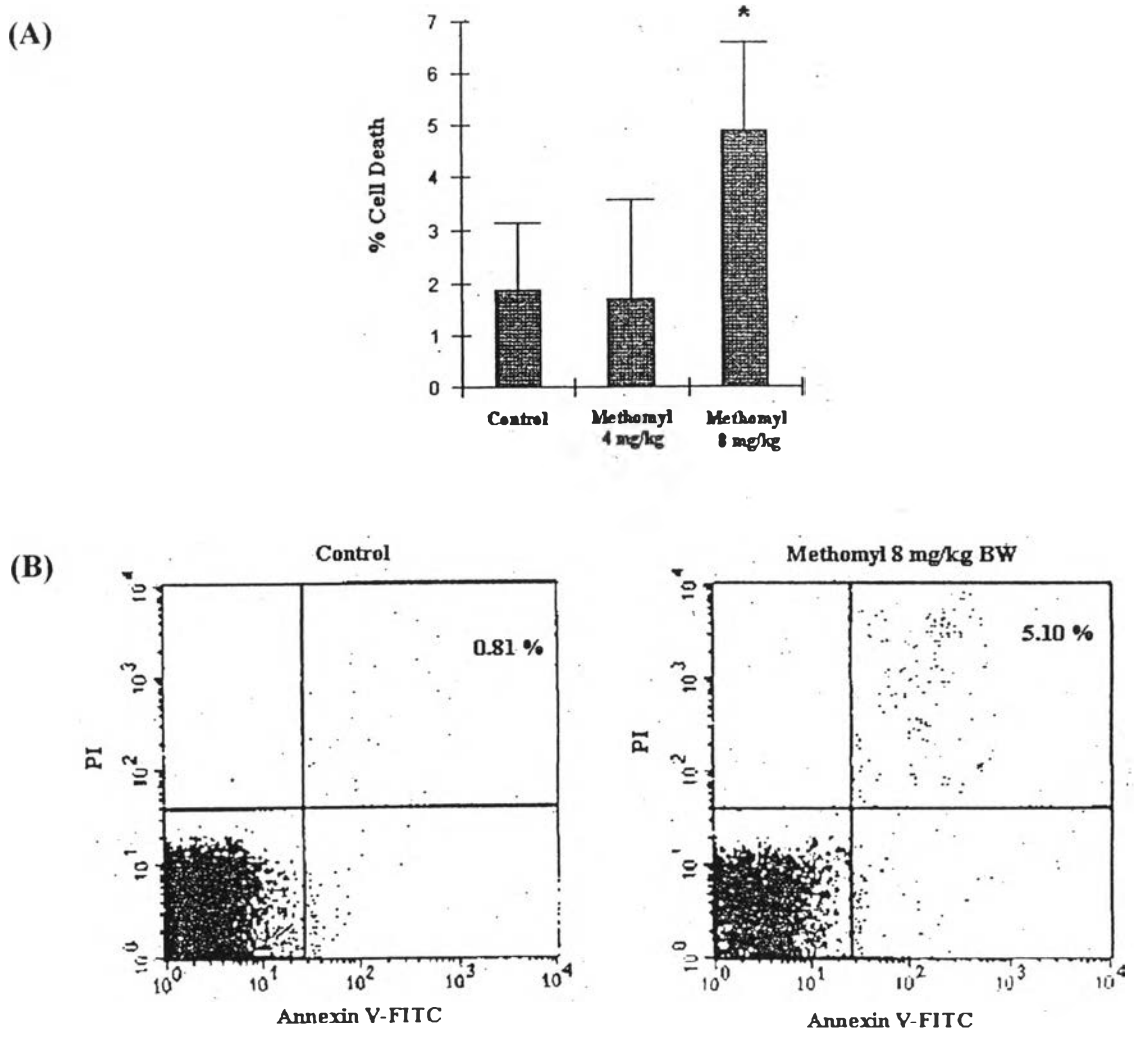

Figure 57 Lymphocytic cell death. (A) Graph shows a significant increase in the death of lymphocytes taken from rats orally exposed to single dose of methomyl at 8 mg/kg body weight (* $p<0.05$); **(B)** Comparison of lymphocytic cell death detected by annexin V-FITC and PI.

Effects of Methomyl on Spleens

(A)

(B)

(C)

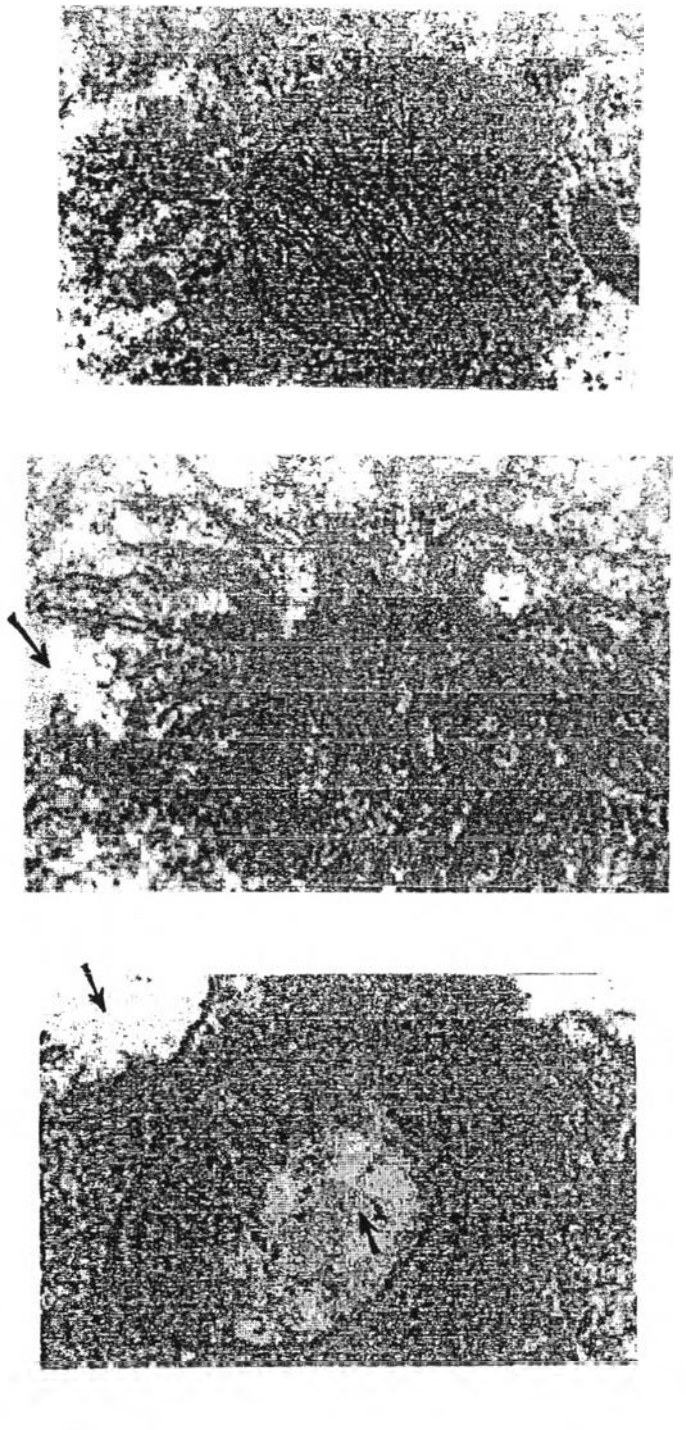

Figure 58 Electron microscopy of spleen cells collected from rats exposed to methomyl. (A) Control; (B) After 6 hours of methomyl treatment at 8 mg/kg body weight (oral, single dose); and (C) After 24 hours of methomyl treatment at 8 mg/kg body weight (oral, single dose). The arrows show mitochondria with cristae loss after methomyl exposure.

Effect of Methomyl on 2,3-DPG Levels in Blood

Table 50 Levels of 2,3-DPG in blood collected from rat after single dose oral-treatments of methomyl for 24 hours. (* $p<0.05$, compared to control)

Methomyl Treatment (mg/kg body weight) (n=6)	Blood Levels of 2,3-DPG (Mean ± S.E.)
Methomyl 0 mg/kg (Control)	3.18 ± 0.321
Methomyl 4 mg/kg	3.46 ± 0.236
Methomyl 8 mg/kg	3.70 ± 0.224*

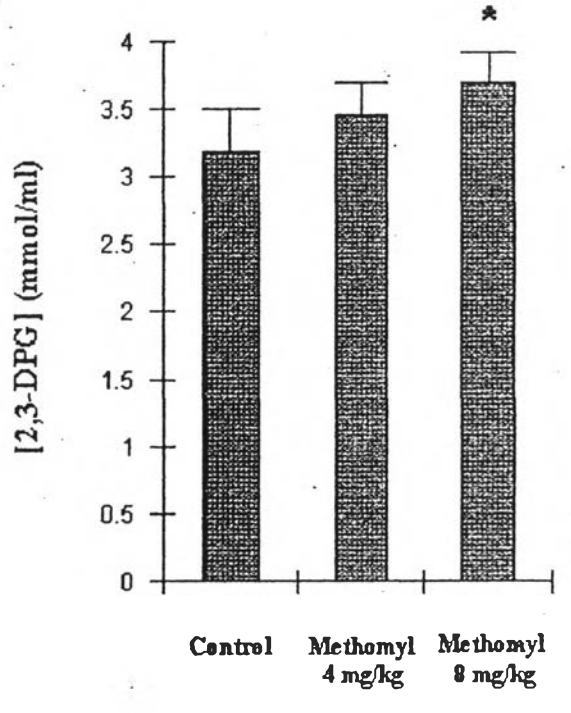

Figure 59 Levels of 2,3-DPG in blood. Graph shows a significant increase in the 2,3-DPG level in blood collected from rats after 24 hour-oral exposure to single dose of methomyl at 8 mg/kg body weight compared to control ($p<0.05$).

Effect of Methomyl on Red Blood Cell NADH-DCIP Reductase Activity

Table 51 NADH-DCIP reductase activities in red blood cells collected from rat after single dose oral-treatments of methomyl for 6 hours. (* $p<0.05$, compared to control)

Methomyl Treatment (mg/kg body weight) (n=6)	Number of folds increased in red blood cell NADH-DCIP reductase activities compared to control (Mean ± S.E.)
Methomyl 0 mg/kg (Control)	1.00 ± 0.000
Methomyl 4 mg/kg	1.151 ± 0.124*
Methomyl 8 mg/kg	1.181 ± 0.175*

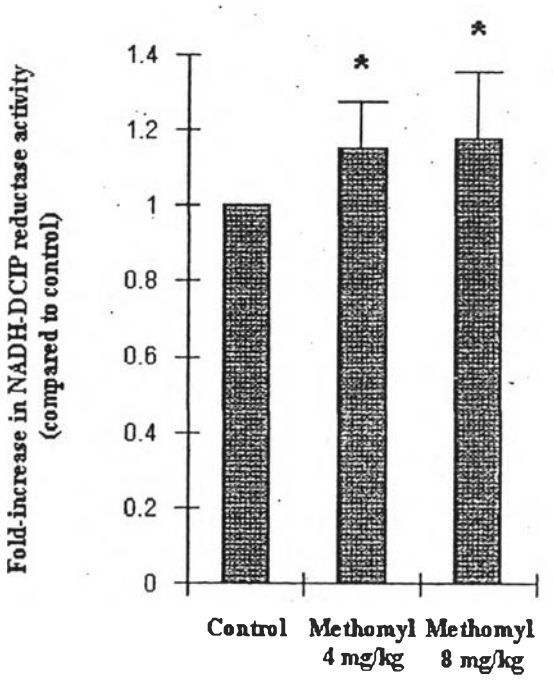

Figure 60 NADH-DCIP reductase activities in red blood cells. Graph shows a significant increase in the NADH-DCIP reductase activities in red blood cells collected from rats after 6 hour-oral exposure to single dose of methomyl at 4 and 8 mg/kg body weight compared to control ($p<0.05$).