CHAPTER IV

RESULTS

The results of this study were divided into three parts as follows:

Part 1: The effects of ethanol-extracted *Pueraria mirifica* root, daidzein, genistein and 17β-estradiol on the proliferation of normal porcine endometrial cells.

Part 2: The effects of ethanol-extracted *Pueraria mirifica* root, daidzein, genistein and 17β-estradiol on the proliferation of RL-95 endometrial cancer cells.

Part 3: To quantitate estrogen receptor protein expression in normal endometrial cells and endometrial cancer cells treated with ethanol-extracted *Pueraria mirifica* root, daidzein, genistein and 17β-estradiol of normal porcine endometrial cells and RL-95 endometrial cancer cells.

Part 1: The effects of ethanol-extracted *Pueraria mirifica* root, daidzein, genistein and 17β-estradiol on the proliferation of normal porcine endometrial cells.

In this experiment, normal porcine endometrial cells (PE-I) harvested from immature female pigs were divided into 4 groups including ethanol-extracted *Pueraria mirifica* root (PM), Genistein (Ge), Daidzein (Di) and 17β- estradiol (E_2) in concentration order of 10^{-11} M, 10^{-10} M, 10^{-9} M, 10^{-8} M, 10^{-7} M, 10^{-6} M, 10^{-5} M or 10^{-4} M. The proliferative effects of drugs were assessed from at least three individual pigs and each concentration was done in duplicate using the MTT colorimetric assay and presented as percent changes from its vehicle control.

1.1 Percent changes from control of normal porcine endometrial cells at 24, 48, 72 hours after treated with *Pueraria mirifica* (PM)

Table 4-1 summarizes the mean percent change from control $\pm$ S.E.M. Cells were treated with PM for 24, 48 or 72 hrs. The results showed that PM at low doses (10^{-11} – 10^{-6} M) had no effect on normal endometrial cells, but at high dose ($>10^{-5}$M) significantly reduced cell numbers of normal endometrial in a dose dependent manner in all time period ($P<0.001$) (Table 4-1 and Figure 4-1).

Table 4-1 Percent changes of numbers from control of normal porcine endometrial cells treated with various concentration of *Pueraria mirifica* (PM) for 24, 48 or 72 hrs.

Log [PM], M	24 hrs	48 hrs	72 hrs
Vehicle (dH_2O)	100.00 ± 0.00^a	100.00 ± 0.00^a	100.00 ± 0.00^a
-11	105.60 ± 7.33^a	92.44 ± 5.78^a	106.2 ± 9.76^a
-10	104.40 ± 5.76^a	99.11 ± 7.04^a	113.70 ± 6.21^a
-9	106.90 ± 5.31^a	101.20 ± 6.05^a	125.30 ± 11.95^a
-8	100.40 ± 6.25^a	106.80 ± 5.04^a	104.00 ± 8.41^a
-7	96.83 ± 4.54^a	110.00 ± 6.11^a	108.90 ± 11.54^a
-6	101.40 ± 3.68^a	107.70 ± 4.54^a	115.90 ± 16.48^a
-5	72.81 ± 5.92^b	76.86 ± 3.57^b	38.44 ± 5.53^b
-4	10.46 ± 1.48^c	8.02 ± 0.35^c	7.70 ± 0.62^c

Data presented as % changes of cells from vehicle control in a value of mean ± S.E.M. Different letters in the same column are significantly different from one another ($P < 0.05$), ANOVA followed by Newman-Keuls Multiple Comparison test.

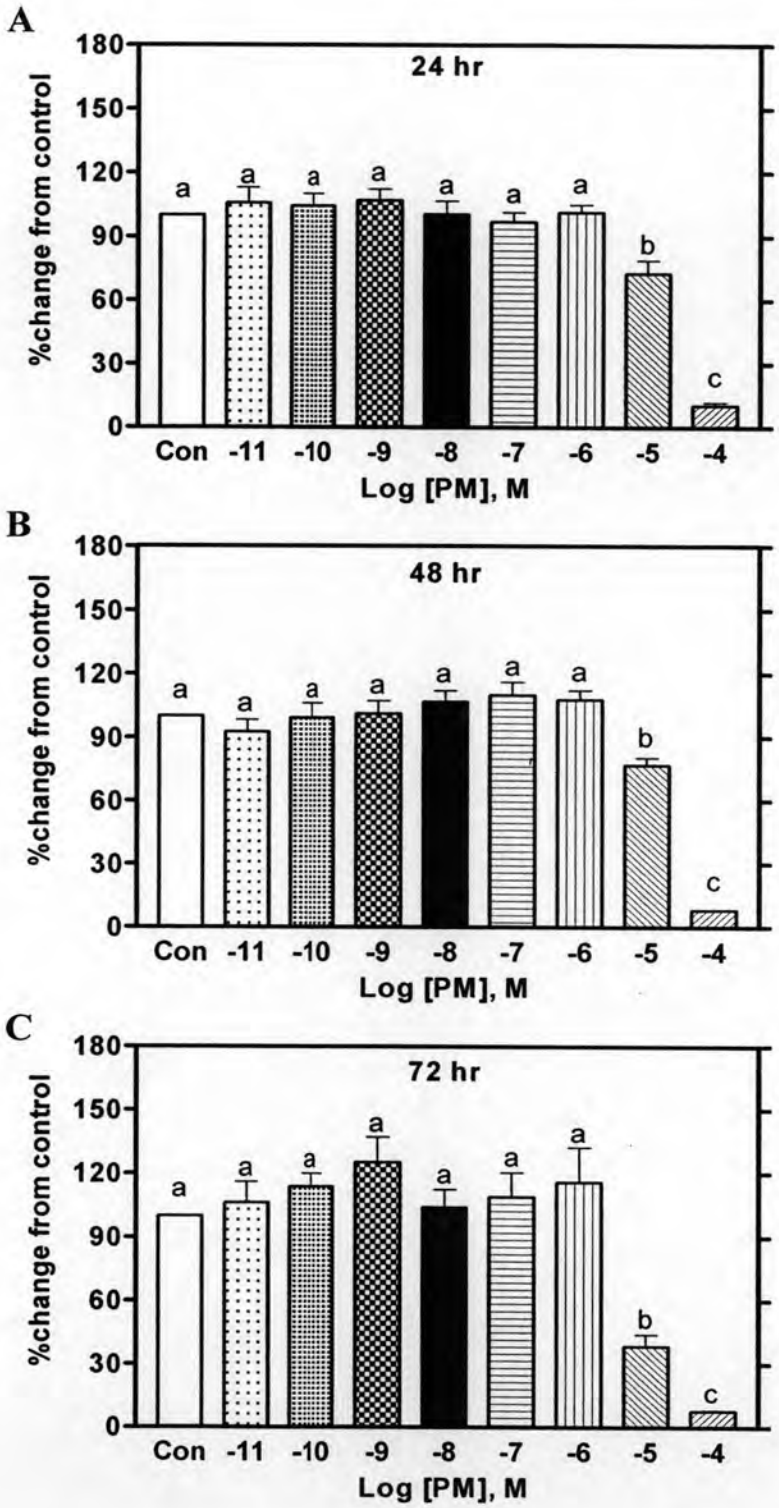

Figure 4-1 Effect of ethanol-extracted *Pueraria mirifica* on the proliferation of normal porcine endometrial cells for a period of A) 24 hrs, B) 48 hrs and C) 72 hrs, determined by MTT proliferation assay. The histograms represent mean ± S.E.M. of a percent proliferation changed of treated cells from control (vehicle). Bars with different letters are significantly different from one another ($P < 0.05$).

1.2 Percent change from control of normal porcine endometrial cells at 24, 48, and 72 hours after treated with daidzein.

Table 4-2 summarizes the mean percent change from control ± S.E.M. Cells were treated with daidzein for 24, 48 or 72 hrs. The results showed that daidzein only at high dose (10^{-4}M) after treated for 72 hrs had significant effect on normal endometrial cells ($P<0.01$) (Table 4-2 and Figure 4-2).

Table 4-2 Percent changes of numbers from control of normal porcine endometrial cells treated with various concentration of daidzein for 24, 48 or 72 hrs.

Log [Di], M	24 hrs	48 hrs	72 hrs
Vehicle (DMSO)	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00[a]
-11	95.84 ± 4.81	90.71 ± 5.68	100.90 ± 4.26[a]
-10	94.11 ± 3.19	85.38 ± 4.18	103.80 ± 5.29[a]
-9	89.92 ± 5.25	91.22 ± 2.99	100.30 ± 4.79[a]
-8	98.73 ± 6.43	95.17 ± 1.78	101.81 ± 4.21[a]
-7	98.60 ± 4.02	92.27 ± 3.79	105.70 ± 4.60[a]
-6	99.64 ± 3.88	91.65 ± 4.45	91.31 ± 5.56[a]
-5	97.34 ± 6.25	89.87 ± 1.53	105.70 ± 5.57[a]
-4	116.2 ± 12.35	86.82 ± 2.69	71.21 ± 6.58[b]

Data presented as mean of % change from control ± S.E.M.

Different letters in the same column are significantly different from one another ($P < 0.01$), ANOVA followed by Newman-Keuls Multiple Comparison test.

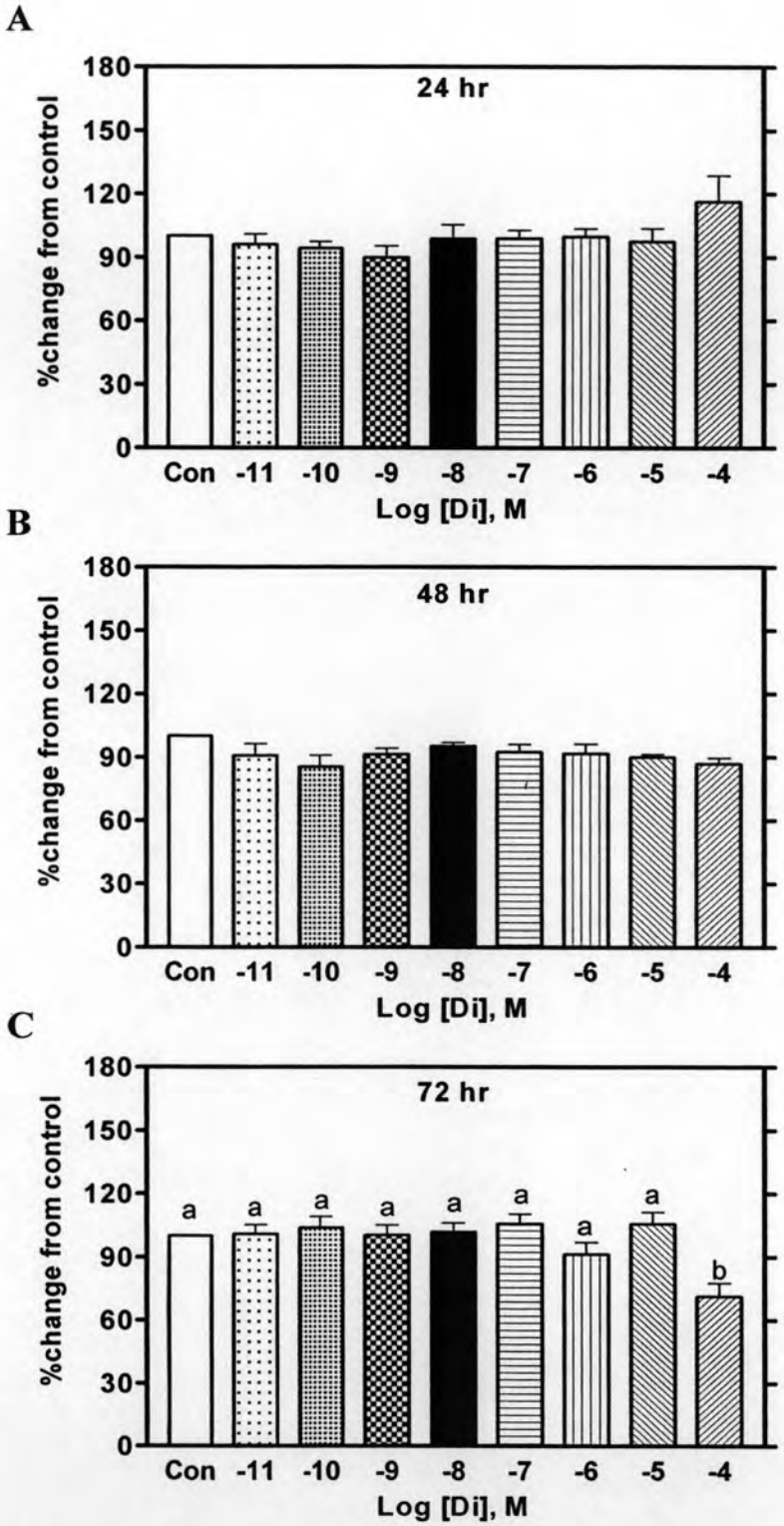

Figure 4-2 Effect of daidzein on the proliferation of normal porcine endometrial cells for a period of A) 24 hrs, B) 48 hrs and C) 72 hrs was determined by MTT proliferation assay. The histograms represent mean ± S.E.M. of a percent proliferation changed of treated cells from control (vehicle) cells. Bars with different letters are significantly different from one another ($P < 0.01$).

1.3 Percent change from control of normal porcine endometrial cells at 24, 48, and 72 hours after treated with genistein.

Table 4-3 summarizes the mean percent change from control $\pm$ S.E.M. Cells were treated with genistein for 24, 48 or 72 hrs. The results showed that at 24 hrs, cells treated with genistein at low doses ($10^{-11} - 10^{-7}$ M) were not different from control. The higher doses ($10^{-6} - 10^{-4}$ M) were different from control and from those treated with 10^{-11} and 10^{-10} M. The highest dose (10^{-4} M) was different from control and those treated with $10^{-11} - 10^{-8}$ M but not $10^{-7} - 10^{-5}$ M. At 48 hrs, the concentration of 10^{-8} M reduced cell numbers when compared to control, lower doses ($10^{-11} - 10\text{-}^{10}$ M) and higher doses ($10^{-6} - 10^{-5}$ M). Similarly, the concentration at 10^{-7} M of genistein had lower cell numbers when compared to control, 10^{-11}, 10^{-10}, 10^{-6}, 10^{-5} M but not different from 10^{-9}M and 10^{-8} M. At the highest concentration (10^{-4} M) of genistein, the number of cells as measured with MTT assay was significantly lower than all other concentrations and this effect was also evident at 72 hrs (Table 4-3 and Figure 4-3).

Table 4-3 Percent changes of numbers from control of normal porcine endometrial cells treated with various concentration of genistein for 24, 48 or 72 hrs.

Log [Ge], M	24 hrs	48 hrs	72 hrs
Vehicle (DMSO)	100.00 ± 0.00	100.00 ± 0.00^{a,b,c}	100.00 ± 0.00^{a}
-11	98.60 ± 5.17	93.84 ± 2.87^{a,b}	93.93 ± 4.47^{a}
-10	108.00 ± 5.59	98.17 ± 3.71^{a,b}	104.20 ± 4.87^{a}
-9	106.80 ± 7.68	92.19 ± 3.89^{b}	94.61 ± 6.34^{a}
-8	108.90 ± 4.55	100.60 ± 3.08^{a,b}	102.00 ± 5.02^{a}
-7	102.10 ± 5.16	92.85 ± 5.04^{a,b}	96.02 ± 7.15^{a}
-6	122.80 ± 6.89	111.00 ± 7.41^{a,b}	108.30 ± 7.48^{a}
-5	124.40 ± 4.84	112.60 ± 5.54^{a,c}	100.30 ± 8.64^{a}
-4	103.00 ± 7.97	69.35 ± 4.87^{d}	46.43 ± 6.49^{b}

Data presented as % changes of cells from control in a value of mean ± S.E.M. Different letters in the same column are significantly different from one another ($P < 0.01$), ANOVA followed by Newman-Keuls Multiple Comparison test.

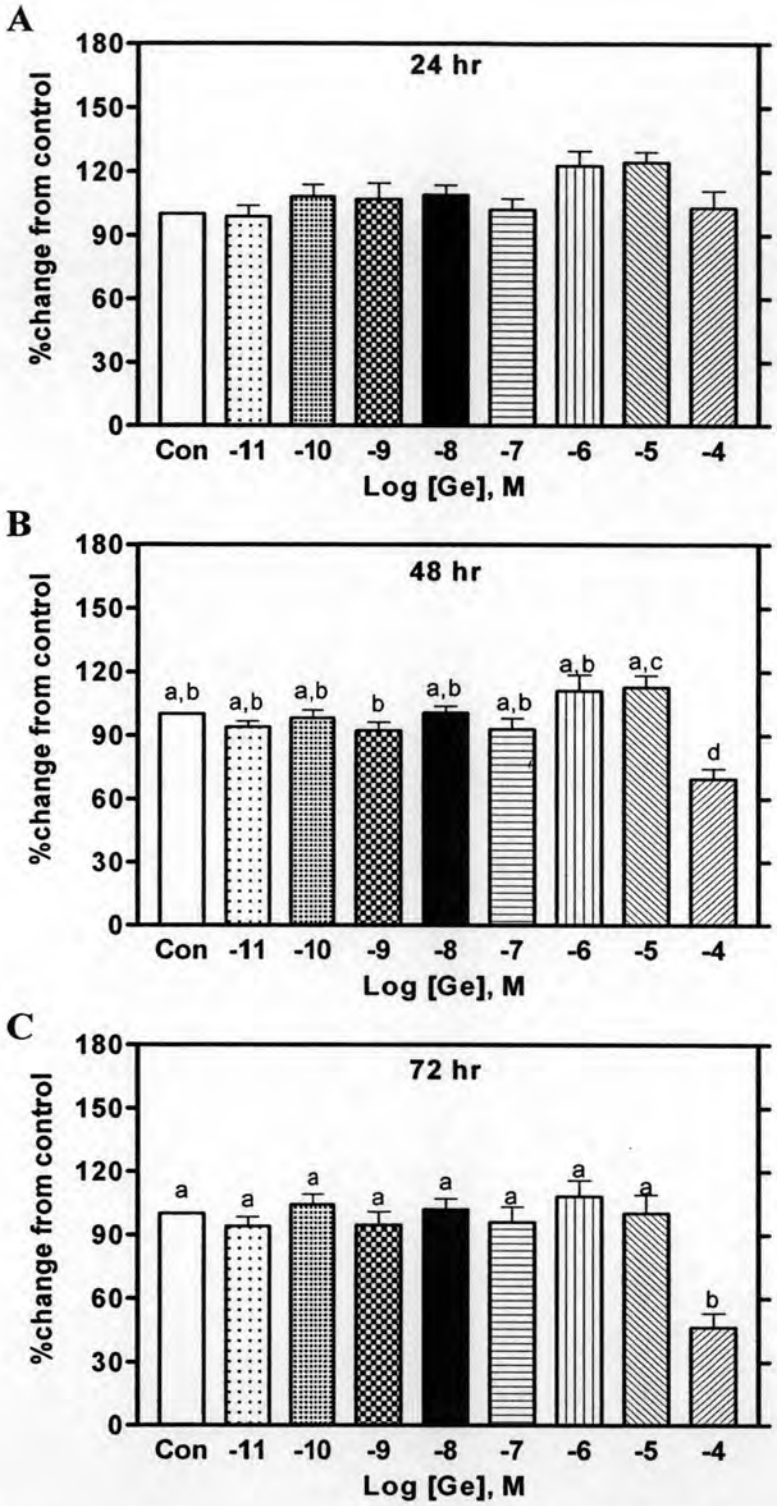

Figure 4-3 Effect of genistein on the proliferation of normal porcine endometrial cells for a period of A) 24 hrs, B) 48 hrs and C) 72 hrs was determined by MTT proliferation assay. The histograms represent mean ± S.E.M. of percent proliferation changes of treated cells from control (vehicle) cells. Bars with different letters are significantly different from one another ($P < 0.05$).

1.4 Percent change from control of normal porcine endometrial cells at 24, 48, and 72 hours after treated with 17β-estradiol (E_2).

Table 4-4 and Figure 4-4 summarize the mean percent change from control ± S.E.M. Cells were treated with 17β-estradiol for 24, 48 or 72 hrs. The results showed that at 24 hrs, 17β-estradiol at low doses (10^{-11} – 10^{-6} M) had no effect on normal endometrial cells. At high doses (10^{-5} and 10^{-4} M) can significantly reduce cell numbers of normal endometrial cell, but the 10^{-5} and 10^{-6} M were not different from one another. The longer incubation, the effects was more pronounced. At 48 and 72 hrs incubation, the E_2 at high doses (10^{-6} – 10^{-4} M) caused a reduction in cell numbers in a dose dependent manner (P<0.001).

Table 4-4 Percent changes of numbers from control of normal porcine endometrial cells treated with various concentration of 17β-estradiol for 24, 48 or 72 hrs.

Log [E_2], M	24 hrs	48 hrs	72 hrs
Vehicle (EtOH)	$100.00 \pm 0.00^{a,b}$	$100.00 \pm 0.00^{a,d}$	100.00 ± 0.00^{a}
-11	121.80 ± 6.88^{a}	$100.70 \pm 2.56^{a,d}$	94.64 ± 3.22^{a}
-10	121.00 ± 8.72^{a}	$97.98 \pm 3.36^{a,d}$	95.57 ± 5.96^{a}
-9	$113.50 \pm 7.70^{a,b}$	$95.50 \pm 3.07^{a,c,d}$	105.00 ± 3.35^{a}
-8	$108.20 \pm 10.38^{a,b}$	$83.96 \pm 2.53^{b,c}$	86.86 ± 2.75^{a}
-7	$99.50 \pm 2.29^{a,b,c}$	88.36 ± 3.46^{c}	95.79 ± 3.55^{a}
-6	$95.86 \pm 4.21^{b,c}$	108.50 ± 1.23^{d}	93.82 ± 4.64^{a}
-5	$96.94 \pm 4.88^{b,c}$	105.60 ± 3.14^{d}	93.19 ± 3.41^{a}
-4	79.24 ± 5.08^{c}	62.30 ± 4.70^{e}	43.41 ± 4.58^{b}

Data presented as % changes of cells from control in a value of mean ± S.E.M. Different letters in the same column are significantly different from one another ($P < 0.05$), ANOVA followed by Newman-Keuls Multiple Comparison test.

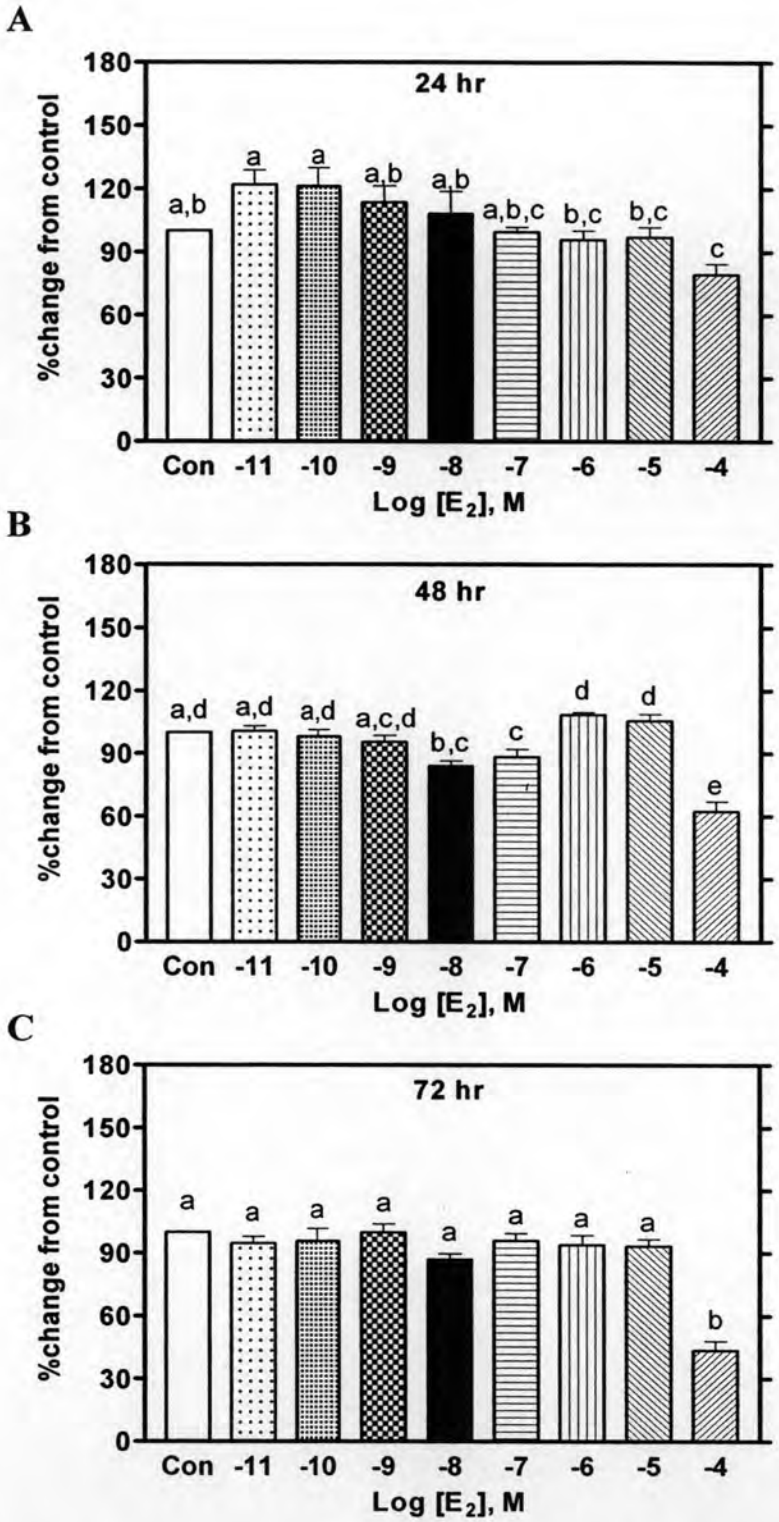

Figure 4-4 Effect of 17β-estradiol on the proliferation of normal porcine endometrial cells for a period of A) 24 hrs, B) 48 hrs and C) 72 hrs was determined by MTT proliferation assay. The histograms represent mean ± S.E.M. of a percent proliferation changed of treated cells from control (vehicle) cells. Bars with different letters are significantly different from one another ($P < 0.05$).

Part 2: The effects of ethanol-extracted *Pueraria mirifica* root, daidzein, genistein and 17β-estradiol on the proliferation of RL-95 endometrial cancer cells.

In similar to PE-I cells, RL-95 cells were divided into 4 experiment groups, including ethanol-extracted *Pueraria mirifica* (PM), daidzein (Di), genistein (Ge) and 17β-estradiol (E_2). Each treatment composed of a concentration order of 10^{-11} M, 10^{-10} M, 10^{-9} M, 10^{-8} M, 10^{-7} M, 10^{-6} M, 10^{-5} M or 10^{-4} M. The proliferative effects of PM, Ge, Di and E_2 on RL-95 were duplicately assessed using the MTT colorimetric assay and evaluated the percent change from control after treated with drug for 24, 48 or 72 hrs.

2.1 Percent changes from control of RL-95 endometrial cancer cells after treated with *Pueraria mirifica* (PM) for 24, 48, and 72 hours

Table 4-5 and Figure 4-5 summarize the mean percent changes from control ± S.E.M. Cancer cells, RL-95 were treated with PM at various concentrations for 24, 48 and 72 hrs. The results revealed that at 24 hrs only at high doses of PM (10^{-5} and 10^{-4} M) can reduce cell numbers as measured with MTT assay. At 48 and 72 hrs, the PM at concentrations of 10^{-6} to 10^{-4} M can reduce cell numbers in a dose dependent manner ($P<0.001$).

Table 4-5 Percent changes of numbers from control of human endometrial cancer cells (RL-95) treated with various concentration of *Pueraria mirifica* for 24, 48 or 72 hrs

Log [PM], M	24 hrs	48 hrs	72 hrs
Vehicle (H_2O)	100.00 ± 0.00^{a}	100.00 ± 0.00^{a}	100.00 ± 0.00^{a}
-11	$89.23 \pm 6.20^{a,b}$	$85.72 \pm 5.54^{a,b}$	108.50 ± 6.20^{a}
-10	$88.89 \pm 13.99^{a,b}$	80.04 ± 6.02^{b}	114.20 ± 5.38^{a}
-9	$87.90 \pm 9.65^{a,b}$	$89.11 \pm 4.81^{a,b}$	97.78 ± 8.56^{a}
-8	96.30 ± 3.32^{a}	$90.46 \pm 3.91^{a,b}$	104.70 ± 7.33^{a}
-7	96.26 ± 3.24^{a}	$85.56 \pm 4.22^{a,b}$	107.10 ± 8.45^{a}
-6	$88.13 \pm 4.34^{a,b}$	62.55 ± 3.81^{c}	75.86 ± 7.28^{b}
-5	64.44 ± 3.82^{b}	28.49 ± 1.17^{d}	24.57 ± 2.03^{c}
-4	8.76 ± 1.08^{c}	4.62 ± 0.40^{e}	3.78 ± 0.24^{d}

Data presented as mean of % change from control ± S.E.M

Different letters in the same column are significantly different from one another ($P < 0.05$), ANOVA followed by Newman-Keuls Multiple Comparison test.

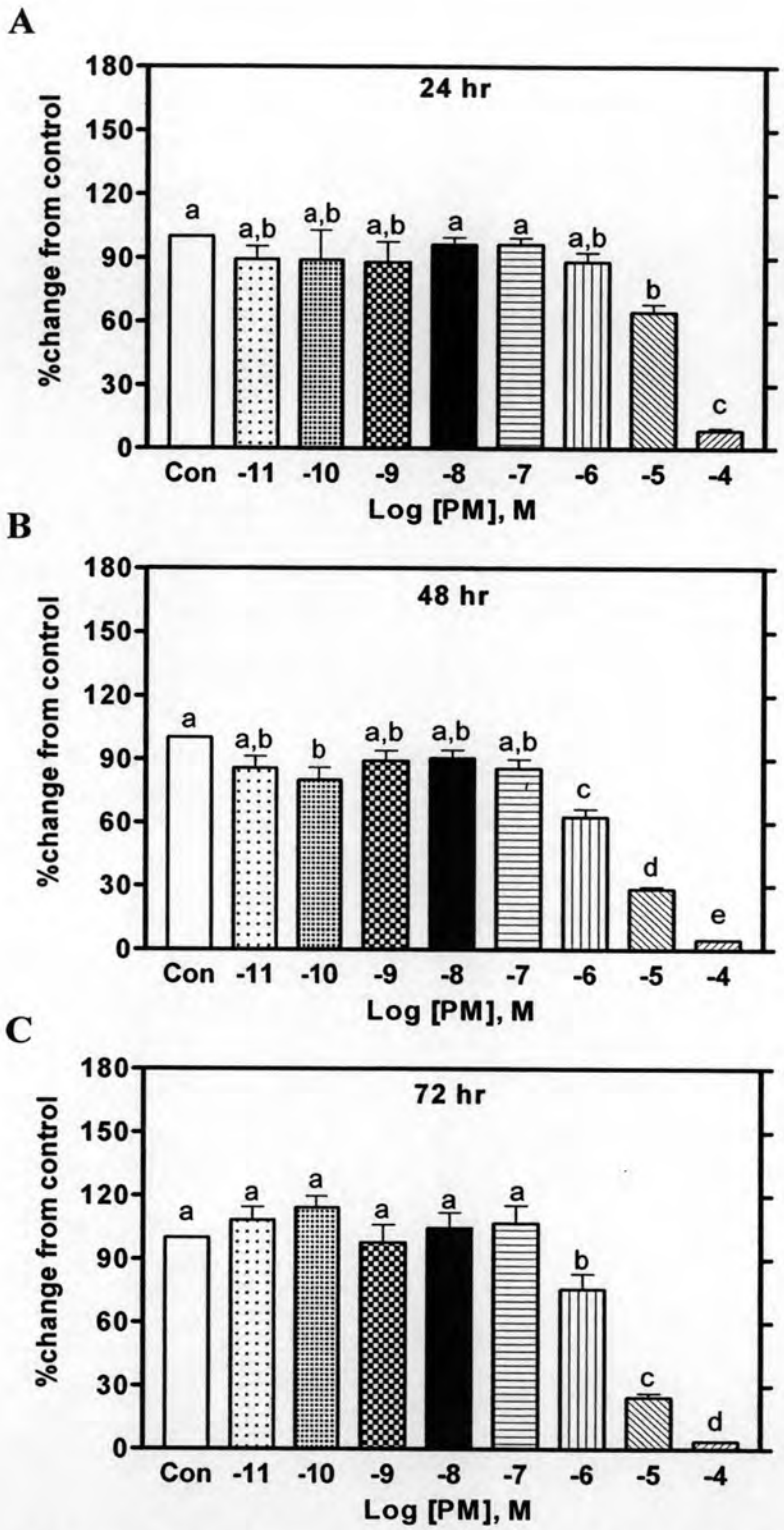

Figure 4-5 Effect of *Pueraria mirifica* on the proliferation of RL-95, endometrial cancer cells for a period of A) 24 hrs, B) 48 hrs and C) 72 hrs was determined by MTT proliferation assay. The histograms represent mean ± S.E.M. of a percent proliferation changed of treated cells from control (vehicle) cells. Bars with different letters are significantly different from one another ($P < 0.05$).

2.2 Percent changes from control of RL-95 endometrial cancer cells after treated with daidzein for 24, 48, and 72 hours

Table 4-6 and Figure 4-6 summarize the mean percent change from control ± S.E.M. Cells were treated with daidzein at various concentrations for 24, 48 and 72 hrs. In this case, the concentration higher than 10^{-8} M was not done since the cost of daidzein was so expensive and we did not observe any effect at the concentration below 10^{-8} M in the preliminary data (n=2 passages). It was therefore designated as not apply (NA) in Figure 4-6. At 24 hour, there was no effect of daidzein on number of cells as assessed with MTT assay. At 48 hrs, the daidzein at 10^{-6} M caused a reduction in cell numbers compared to vehicle control but was not different from 10^{-8}, 10^{-7} or 10^{-5} M. The highest concentration of daidzein (10^{-4} M) demonstrated a significantly lower number of cells than control and other concentrations at 48 and 72 hours.

Table 4-6 Percent changes of numbers from control of human endometrial cancer cells (RL-95) treated with various concentration of daidzein for 24, 48 or 72 hrs.

Log [Di], M	24 hrs	48 hrs	72 hrs
Vehicle (DMSO)	100.00 ± 0.00	100.00 ± 0.00^{a}	100.00 ± 0.00^{a}
-8	96.34 ± 6.74	91.02 ± 7.01^{a,b}	109.10 ± 3.67^{a}
-7	90.68 ± 6.40	83.19 ± 2.23^{a,b}	100.60 ± 3.77^{a}
-6	91.29 ± 2.09	78.30 ± 5.53^{b}	105.00 ± 6.32^{a}
-5	94.30 ± 8.67	88.38 ± 4.21^{a,b}	104.10 ± 1.59^{a}
-4	77.79 ± 13.80	52.92 ± 6.15^{c}	62.47 ± 12.92^{b}

Data presented as mean of % change from control ± S.E.M

Different letters in the same column are significantly different from one another ($P < 0.001$), ANOVA followed by Newman-Keuls Multiple Comparison test.

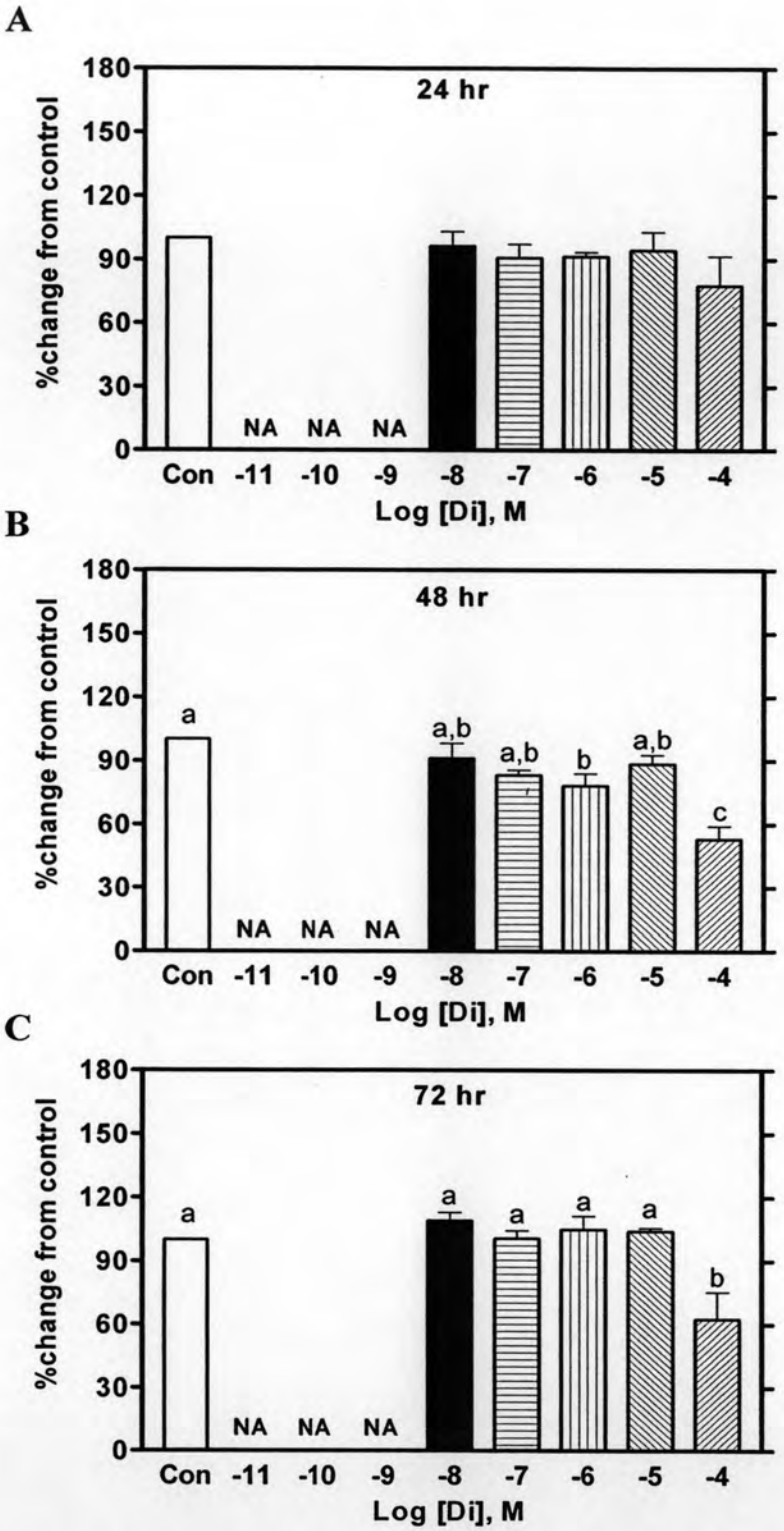

Figure 4-6 Effect of daidzein on the proliferation of RL-95, endometrial cancer cells for a period of A) 24 hrss, B) 48 hrs and C) 72 hrs was determined by MTT proliferation assay. The histograms represent mean ± S.E.M. of a percent proliferation changed of treated cells from control (vehicle) cells. Bars with different letters are significantly different from one another ($P < 0.05$). NA = not apply.

2.3 Percent changes from control of RL-95 endometrial cancer cells after treated with genistein for 24, 48, and 72 hours

Table 4-7 and Figure 4-7 summarize the mean percent changes from control ± S.E.M. Cells were treated with genistein at various concentrations for 24, 48 and 72 hrs. In similar to the result of daidzein, the concentration lower than 10^{-8} M was not completely done in three passages of RL-95 because of the same reason given in 2.2. However, the highest concentration of genistein (10^{-4} M) demonstrated a significantly lower number of cells than control and other concentrations at 48 and 72 hours.

Table 4-7 Percent changes of numbers from control of human endometrial cancer cells (RL-95) treated with various concentration of genistein for 24, 48 or 72 hrs.

Log [Ge], M	24 hrs	48 hrs	72 hrs
Vehicle (DMSO)	100.00 ± 0.00	100.00 ± 0.00[a]	100.00 ± 0.00[a]
-8	107.50 ± 4.44	87.38 ± 10.36[a,b]	82.67 ± 9.39[a]
-7	106.20 ± 7.37	99.93 ± 12.92[a]	95.66 ± 6.66[a]
-6	100.60 ± 7.93	96.20 ± 9.27[a]	101.30 ± 10.08[a]
-5	104.20 ± 1.38	85.89 ± 13.61[a,b]	82.90 ± 14.25[a]
-4	86.61 ± 4.22	52.27 ± 7.74[b]	41.96 ± 5.38[b]

Data presented as mean of % change from control ± S.E.M

Different letters in the same column are significantly different from one another ($P < 0.001$), ANOVA followed by Newman-Keuls Multiple Comparison test.

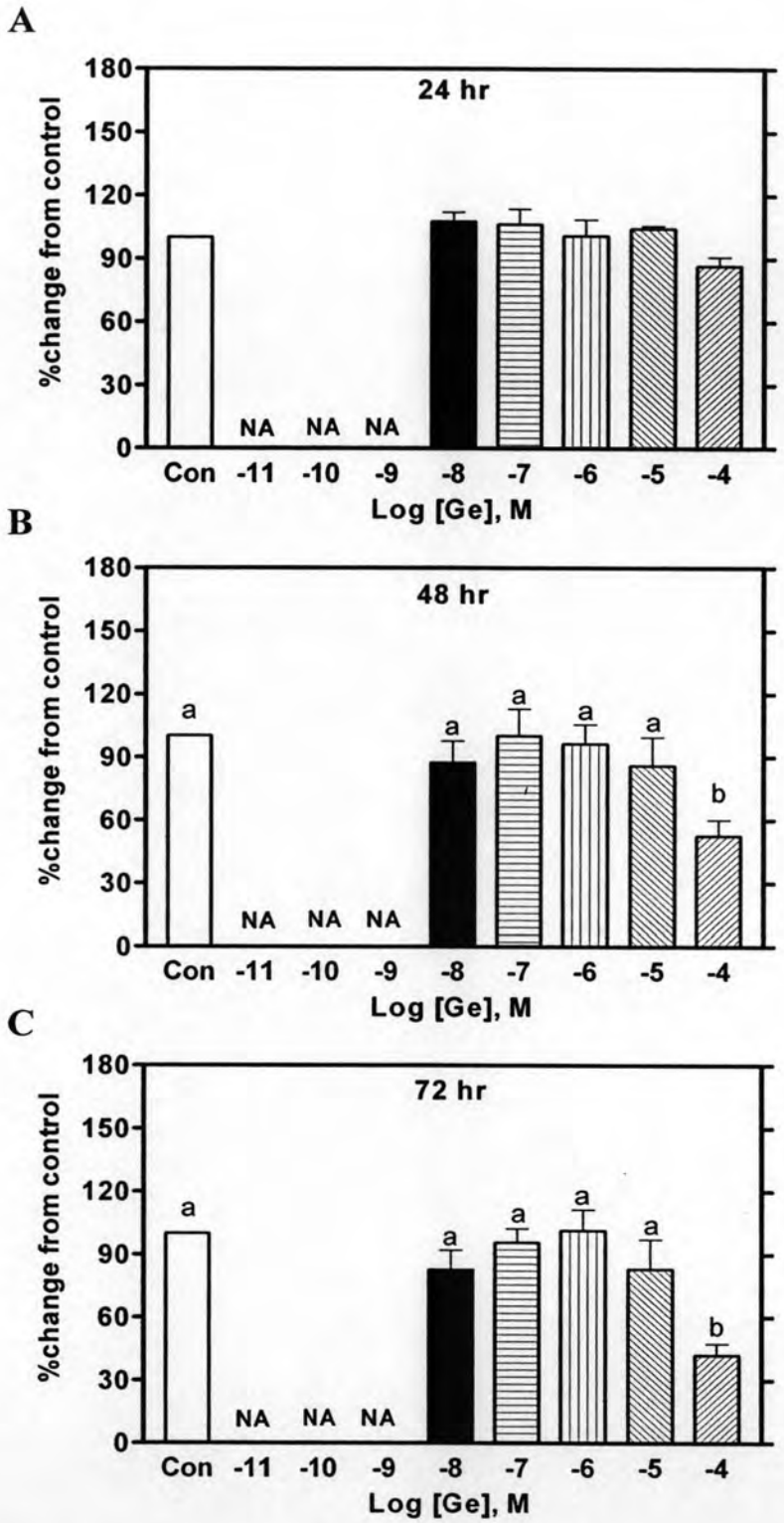

Figure 4-7 Effect of Genistein on the proliferation of RL-95, endometrial cancer cells for a period of A) 24 hrs, B) 48 hrs and C) 72 hrs was determined by MTT proliferation assay. The histograms represent mean ± S.E.M. of a percent proliferation changed of treated cells from control (vehicle) cells. Bars with different letters are significantly different from one another ($P < 0.05$). NA = not apply.

2.4 Percent changes from control of RL-95 endometrial cancer cells after treated with 17β-estradiol for 24, 48, and 72 hour.

Table 4-8 and Figure 4-8 summarize the mean percent change from control ± S.E.M. Cells were treated with 17β-estradiol at various concentrations for 24, 48 and 72 hrs. At 24 hour, there was no effect of 17β-estradiol on number of cells as assessed with MTT assay. The highest concentration of 17β-estradiol (10^{-4} M) demonstrated a significantly lower number of cells than control and other concentrations at 48 and 72 hours.

Table 4-8 Percent changes from control of RL-95 endometrial cancer cells were treated 17β-estradiol (E_2) at 24, 48, 72 hrs.

Log [E_2], M	24 hrs	48 hrs	72 hrs
Vehicle (EtOH)	100.00 ± 0.00	100.00 ± 0.00[a]	100.00 ± 0.00[a]
-11	104.20 ± 13.18	103.80 ± 5.45[a]	105.90 ± 6.32[a]
-10	107.60 ± 8.00	102.20 ± 6.19[a]	109.40 ± 6.78[a]
-9	109.60 ± 9.47	106.40 ± 4.31[a]	99.99 ± 2.34[a]
-8	103.00 ± 9.92	102.60 ± 7.54[a]	102.20 ± 4.31[a]
-7	106.20 ± 11.92	100.30 ± 9.08[a]	106.80 ± 5.67[a]
-6	106.80 ± 11.11	109.00 ± 6.82[a]	109.50 ± 6.02[a]
-5	100.30 ± 5.81	102.30 ± 6.59[a]	105.50 ± 6.92[a]
-4	74.02 ± 5.16	47.47 ± 3.17[b]	31.34 ± 6.52[b]

Data presented as mean of % change from control ± S.E.M

Different letters in the same column are significantly different from one another ($P < 0.001$), ANOVA followed by Newman-Keuls Multiple Comparison test.

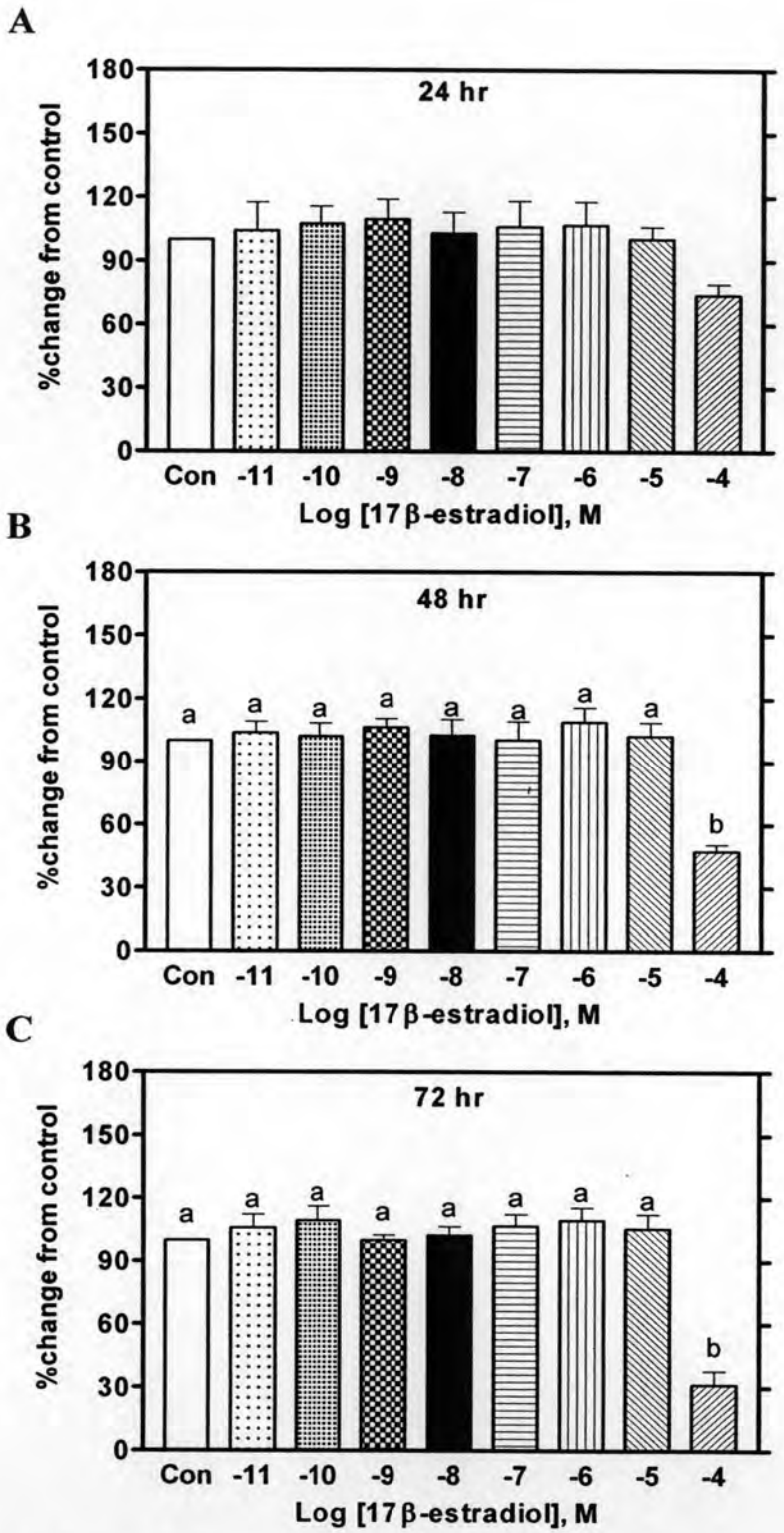

Figure 4-8 Effect of 17β-estradiol on the proliferation of RL-95, endometrial cancer cells for a period of A) 24 hrs, B) 48 hrs and C) 72 hrs was determined by MTT proliferation assay. The histograms represent mean ± S.E.M. of a percent proliferation changed of treated cells from control (vehicle) cells. Bars with different letters are significantly different from one another ($P < 0.05$).

Part 3: To quantitate estrogen receptor protein expression in normal endometrial cells and endometrial cancer cells treated with ethanol-extracted *Pueraria mirifica* root, daidzein, genistein and 17β-estradiol.

In regard to many reports of differences of the heterogeneity of ER-α and ER-β in normal cells and cancer cells, we used the Western blot analysis to investigate the levels of estrogen receptor protein expression using anti-human ER-α or ER-β antibodies. The protein from either PE-I or RL-95 treated with 2 selected concentrations of drugs for 72 hrs were chosen; at this time point, the changed in protein levels was believed to be stable. The concentrations selected were based on the results from part 1 and part 2. For PM, the concentrations of 10^{-9} and 10^{-6} M were selected; since 10^{-6} M was the lowest concentration affected cell proliferation while at 10^{-9} M had no effect on proliferation. Moreover, as we cannot rule out that at 10^{-4} M of PM, the reduction in cell numbers was due to PM or osmotic effect (as H_2O was used as a vehicle). This proliferative effect was evident on the cancer cells RL-95, therefore it was interesting to correlate this anti-proliferative effect on receptor levels. In order to compare with normal endothelial cells, similar concentration of PM was then selected for PE-I. For daidzein, genistein and 17β-estradiol, the selected concentrations were 10^{-9} and 10^{-4} M; since 10^{-4} M was the only dose affected cell proliferation in both cell types and 10^{-9} M had no effect and was used for comparison. The preliminary of the positive control for ER-α or ER-β were respectively determined with MCF-7 and rat ovary in the present study (data not shown). The positive band of ER-α or ER-β was detected at 53 kD or 66 kD, respectively. Semi-quantitative of estrogen protein levels were calculated from the ratio of the density of ER to the density of β-actin which is the structural protein found in both PE-I and RL-95. In addition, the ratio of normalized ER-β to ER-α density was also considered in all cells treated with or without drugs.

3.1 Estrogen receptor protein level in normal porcine endometrial cell (PE-I) and human endometrial cancel cell (RL-95)

Focusing on the different levels of ER-α or ER-β based on Western blot analysis, full length ER-α and ER-β proteins were detected in both normal and cancer cells but at different levels. In normal porcine endometrial cell (n > 5 pigs), the ER-α levels were significantly higher than ER-β by 3 fold (Table 4-9 and Figure 4-9A and B). The results of ER levels were opposite in the human endometrial cancer cells which revealed ER-β level > ER-α level by 16 fold (Table 4-9 and Table 4-10). Comparing between normal cells and cancer cells, ER-α of normal endometrial cell (2.81 ± 0.53) was significantly higher than those of RL-95 (0.18 ± 0.11, $P<0.05$). However, there was no significant different in ER-β level of normal and cancer cell (0.74 ± 0.12 and 2.15 ± 0.18, respectively). Interestingly, the ratio of ER-β to ER-α of endometrial cancer cell (RL-95, 16.81 ± 0.45) was dramatically higher than those of normal endometrial cells (PE-I, 0.31 ± 0.09).

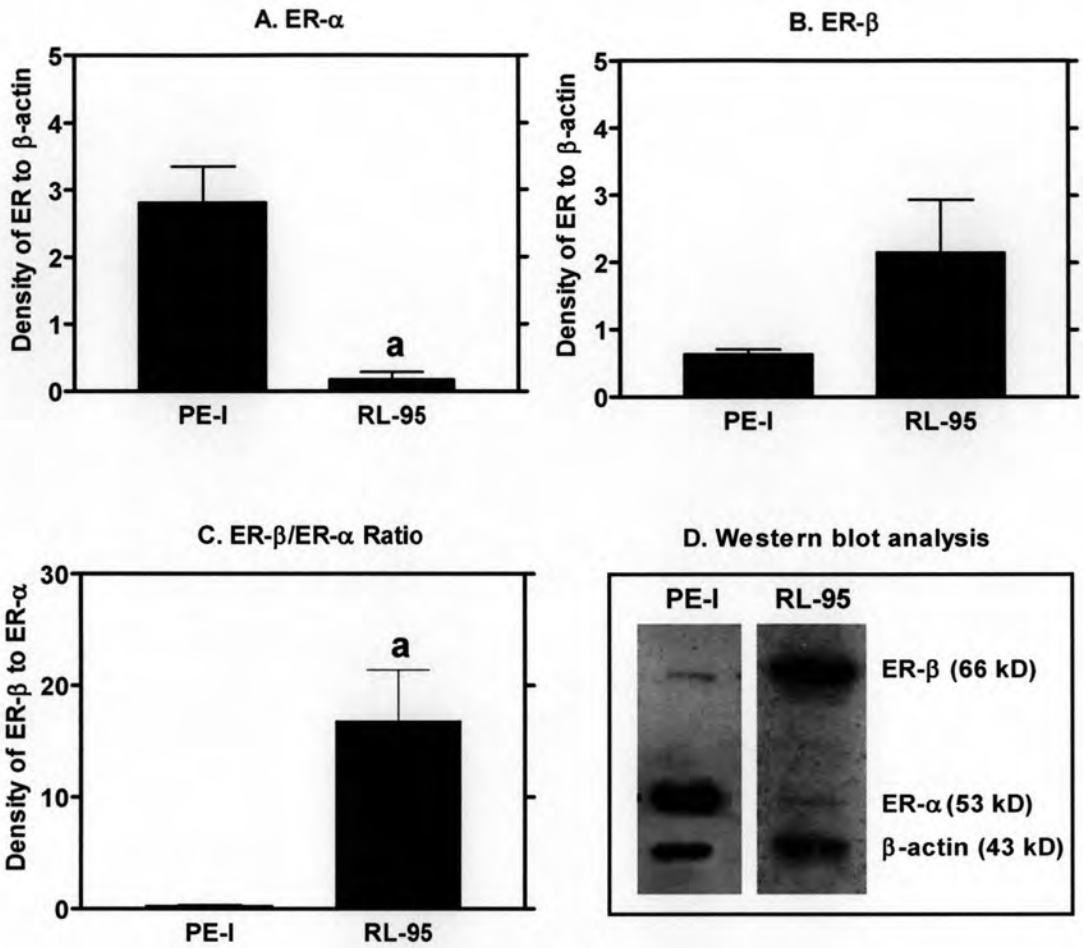

Figure 4-9 Histograms (A-C) illustrate mean ± S.E.M of estrogen receptor protein levels of normal porcine endometrial cells (PE-I) and endometrial cancer cells (RL-95-2) grew in DMEM supplementation with 2%FBS for 48 hrs calculated from Western blot technique. A) ratio of ER-α/β-actin, B) ratio of ER-β/β-actin and C) ratio of ER-β/ER-α. [a] is significantly different between PE-I and RL-95 by student unpaired *t*-test (n= 3-5 pigs or passages, $P<0.05$). Photograph D) represents band of estrogen receptor α-and β- protein and β-actin proteins from Western blot technique.

3.2 Estrogen receptor pretein level in normal porcine endometrial cell (PE-I) treated with ethanol-extracted *Pueraria mirifica* root, daidzein, genistein or 17β-estradiol

Table 4-9 summarizes the mean of densitometry ratio of ER to β-actin ± S.E.M. The ER-α, ER-β and ratio of ER-β /ER-α density of cell treated with dH_2O were compared to those of DMSO or ethanol (EtOH) which are respectively a vehicle for Ge and Di, or 17β -estradiol. Even though either ER-α or ER-β density was not significantly differences from control, the ratio of ER-β/ER-α density of normal endometrial cell in the presence of those vehicles were significantly higher than those of control (Table 4-9). In the present study, normal endometrial cells treated with PM at low concentration (10^{-9} M) revealed the reduction of both ER-α and ER-β but not the ratio of ER-β /ER- α density (Table 4-9 and Figure 4-10). In contrast the PM at higher concentration of 10^{-6} M increased the ratio of ER-β /ER- α density to 1.32 ± 0.20 (Table 4-9 and Figure 4-10). However, there was no significant change of ER-α, ER-β and ratio of ER-β /ER-α density of cell treated with daidzein, genistein or 17β -estradiol from those of its vehicle control (Table 4-9 and Figure 4-11 to 4-13).

Table 4-9 Ratio of ER alpha and ER beta density of normal porcine endometrial cells (PE-I)

Drug treatment	Raio of ER alpha/ beta actin	Ratio of ER beta/ beta actin	Ratio of ER beta/ER alpha
Vehicle			
dH_2O	2.81 ± 0.53	0.74 ± 0.12	0.31 ± 0.09
DMSO	2.32 ± 1.68	2.35 ± 1.85	1.72 ± 0.35[A]
EtOH	1.27 ± 0.37	1.30 ± 0.60	1.05 ± 0.30[A]
***Peuraria mirifica* (PM)**			
10^{-9} M	1.02 ± 0.27[a]	0.27 ± 0.09[a]	0.54 ± 0.20
10^{-6} M	2.38 ± 1.17	2.73 ± 2.72	1.32 ± 0.20[a]
Daidzein (Di)			
10^{-9} M	1.76 ± 1.44	1.80 ± 1.51	0.86 ± 0.45
10^{-4} M	2.05 ± 1.64	2.31 ± 1.71	1.73 ± 0.56
Genistein (Ge)			
10^{-9} M	3.26 ± 2.58	2.35 ± 1.85	0.71 ± 0.42
10^{-4} M	4.06 ± 3.10	4.38 ± 3.77	1.37 ± 0.58
17β-estradiol (E_2)			
10^{-9} M	1.73 ± 0.50	1.36 ± 0.57	1.00 ± 0.28
10^{-4} M	1.90 ± 0.52	2.35 ± 0.55	1.63 ± 0.45

Data presented as mean ± S.E.M of estrogen protein level of normal porcine endometrial cell (PE-I, n = 3-5 pigs)

[A] Represents significant different between DMSO or EtOH vs. dH_2O at $P < 0.05$, student unpaired t-test; [a] Represents significant different from its vehicle (PM vs dH_2O; Di or Ge DMSO and E_2 vs EtOH) at $P < 0.05$, student unpaired t-test.

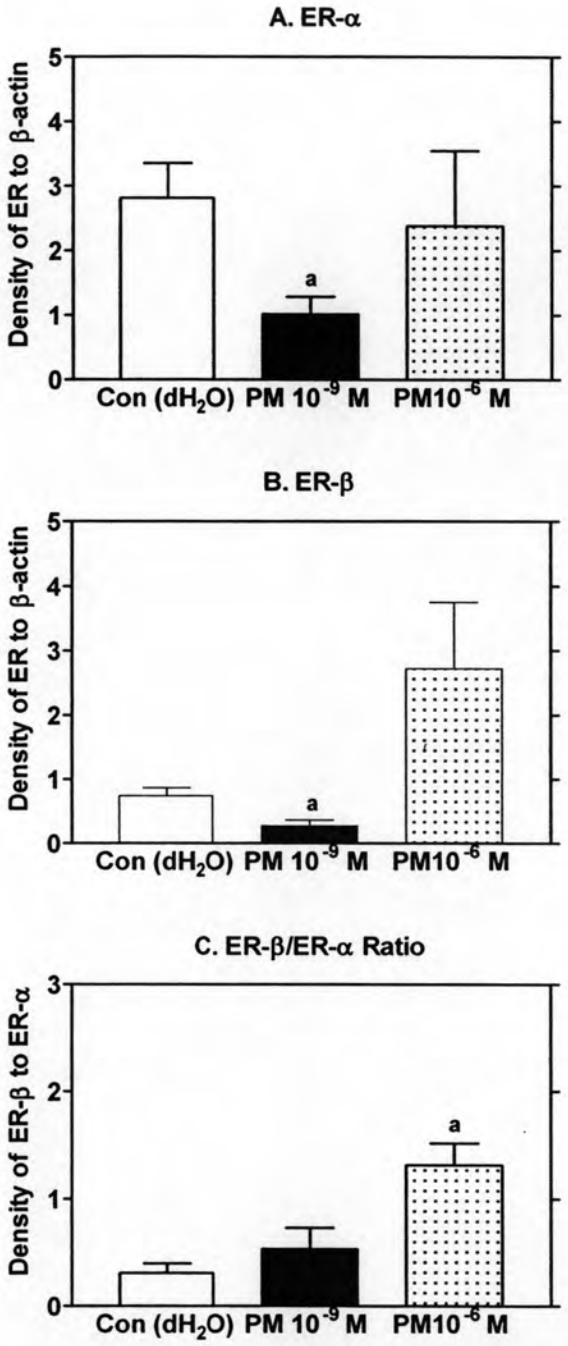

Figure 4-10 Histograms illustrate mean ± S.E.M of estrogen receptor protein levels of normal porcine endometrial cells (PE-I) treated with *Pueraria mirifica*. A) ratio of ER-α/β-actin, B) ratio of ER-β/β-actin and C) ratio of ER-β/ER-α. [a] are significantly different from vehicle by student unpaired *t*-test ($P < 0.05$).

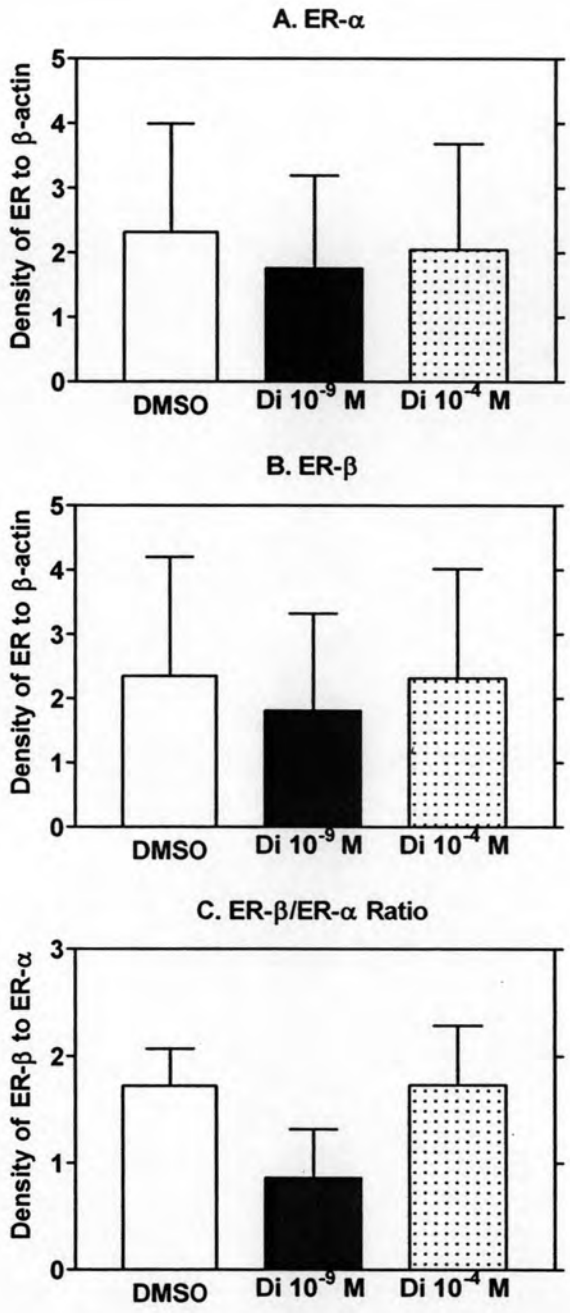

Figure 4-11 Histograms illustrate mean ± S.E.M of estrogen receptor protein levels of normal porcine endometrial cells (PE-I) treated with daidzein. A) ratio of ER-α/β-actin, B) ratio of ER-β/β-actin and C) ratio of ER-β/ER-α. [a] are significantly different from vehicle by student unpaired *t*-test ($P < 0.05$).

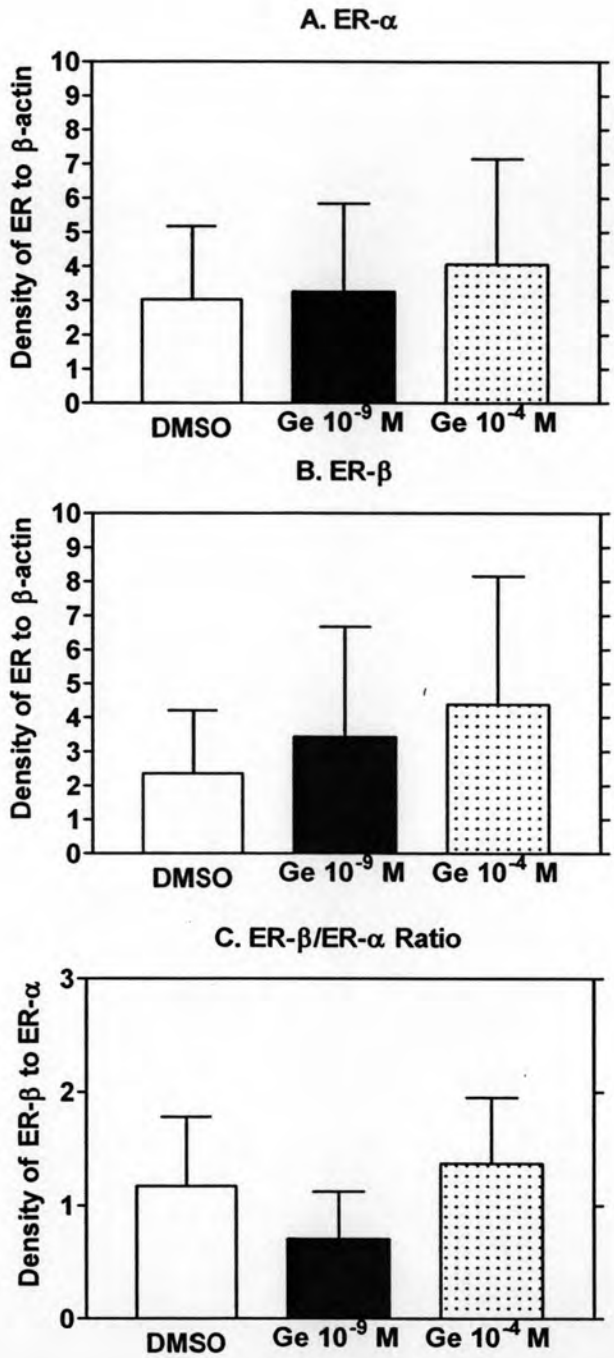

Figure 4-12 Histograms illustrate mean ± S.E.M of estrogen receptor protein levels of normal porcine endometrial cells (PE-I) treated with genistein. A) ratio of ER-α/β-actin, B) ratio of ER-β/β-actin and C) ratio of ER-β/ER-α. [a] are significantly different from vehicle by student unpaired *t*-test ($P < 0.05$).

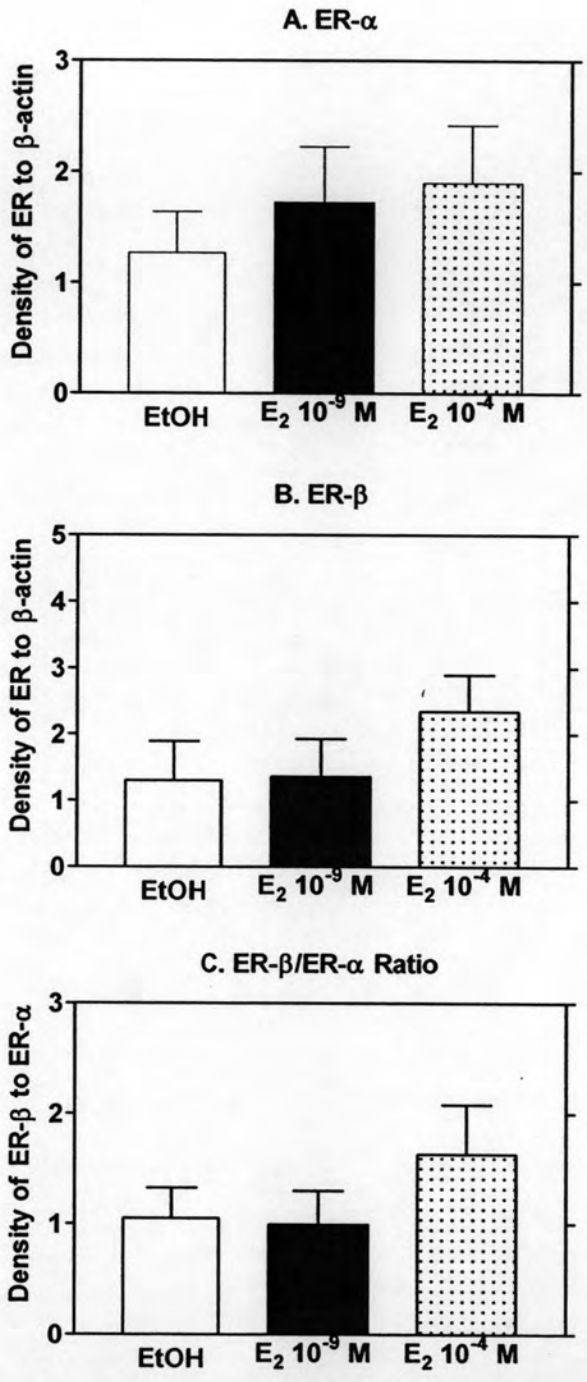

Figure 4-13 Histograms illustrate mean ± S.E.M of estrogen receptor protein levels of normal porcine endometrial cells (PE-I) treated with 17β-estradiol. A) ratio of ER-α/β-actin, B) ratio of ER-β/β-actin and C) ratio of ER-β/ER-α. [a] are significantly different from vehicle by student unpaired *t*-test ($P < 0.05$).

3.3 Estrogen receptor protein level in human endometrial cancer cell (RL-95) treated with ethanol-extracted *Pueraria mirifica* root, daidzein, genistein or 17β-estradiol

Table 4-10 and Figure 4-14 summarize the mean of densitometry ratio of ER to β-actin ± S.E.M. The ER-α, ER-β and ratio of ER-β /ER-α density of cell treated with dH_2O were compared to those of DMSO or ethanol (EtOH) which are respectively a vehicle for Ge and Di, or 17β -estradiol. Consistent to the results of normal endometrial cell, the ratio of ER-β/ER-α density but not ER-α or ER-β density of normal endometrial cell in the presence of DMSO or EtOH vehicles were significantly higher than those of control (Table 4-10). In contrast to normal endometrial cells treated with PM at low concentration (10^{-9} M) did not reveal the reduction of both ER-α and ER-β or the ratio of ER-β /ER-α density (Table 4-10 and Figure 4-14). In contrast, the PM at higher concentration of 10^{-6} M increased the ER-α density (1.04 ± 0.21) with the reduction of ratio of ER-β /ER-α density to 1.26 ± 0.35 (Table 4-10 and Figure 4-14). There were no significant changes of ER-α, ER-β and ratio of ER-β /ER-α density of cell treated with daidzein, genistein or 17β -estradiol from those of its vehicle control (Table 4-10 and Figure 4-14 to 4-17).

Table 4-10 Ratio of ER alpha and ER beta of human endometrial cancer cells (RL-95)

Drug treatment	Raio of ER alpha/ beta actin	Ratio of ER beta/ beta actin	Ratio of ER beta/ER alpha
Vehicle			
dH_2O	0.18 ± 0.11	2.15 ± 0.78	16.80 ± 4.50
DMSO	0.21 ± 0.06	1.30 ± 0.56	3.09 ± 1.55[A]
EtOH	0.57 ± 0.15	1.60 ± 0.70	2.19 ± 0.30[A]
***Peuraria mirifica* (PM)**			
10^{-9} M	0.18 ± 0.094	0.89 ± 0.52	3.99 ± 1.90
10^{-6} M	1.04 ± 0.21[a]	1.10 ± 0.65	1.26 ± 0.35[a]
Daidzein (Di)			
10^{-9} M	0.56 ± 0.10	0.89 ± 0.52	1.91 ± 1.25
10^{-4} M	0.56 ± 0.13	1.15 ± 0.65	2.52 ± 1.72
Genistein (Ge)			
10^{-9} M	0.35 ± 0.11	0.90 ± 0.69	4.64 ± 3.91
10^{-4} M	0.31 ± 0.31	1.30 ± 0.77	0.16 ± 0.16
17β-estradiol (E_2)			
10^{-9} M	0.33 ± 0.17	2.40 ± 0.98	5.17 ± 1.95
10^{-4} M	0.13 ± 0.08	2.75 ± 1.71	6.56 ± 2.08

Data presented as mean ± S.E.M of estrogen protein level of human endometrial cancer cells (RL-95, n = 3 passages)

[A] Represent significant different between DMSO or EtOH vs. dH_2O at $P < 0.05$, student unpaired t-test; [a] Represent significant different from its vehicle (PM vs dH_2O; Di or Ge DMSO and E2 vs. EtOH) at $P < 0.05$, student unpaired t-test.

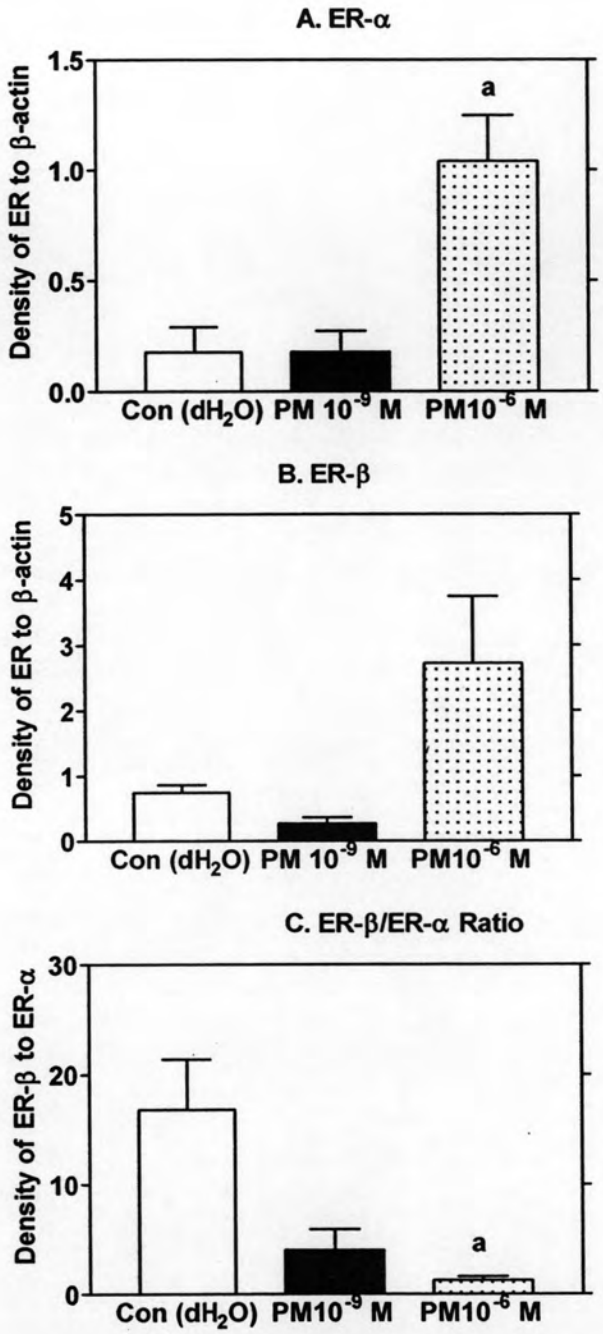

Figure 4-14 Histograms illustrate mean ± S.E.M of estrogen receptor protein levels of human endometrial cancer cells (RL-95) treated with *Pueraria mirifica*. A) ratio of ER-α/β-actin, B) ratio of ER-β/β-actin and C) ratio of ER-β/ER-α. [a] are significantly different from vehicle by student unpaired *t*-test ($P < 0.05$).

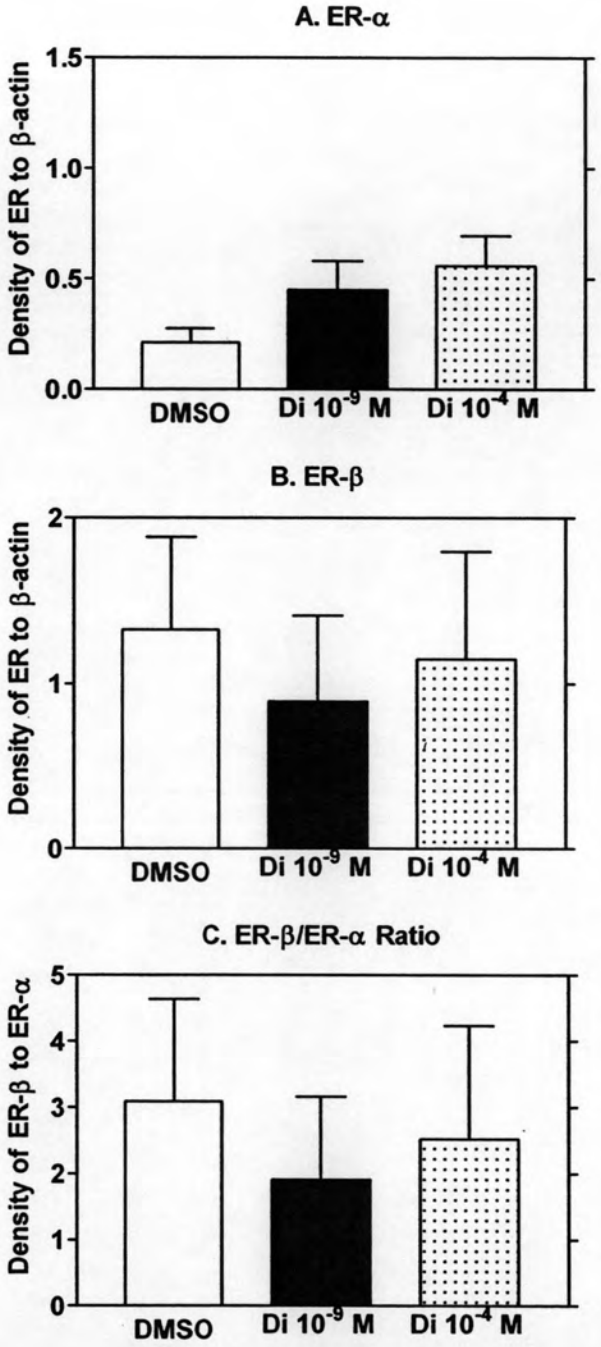

Figure 4-15 Histograms illustrate mean ± S.E.M of estrogen receptor protein levels of human endometrial cancer cells (RL-95) treated with daidzein. A) ratio of ER-α/β-actin, B) ratio of ER-β/β-actin and C) ratio of ER-β/ER-α.

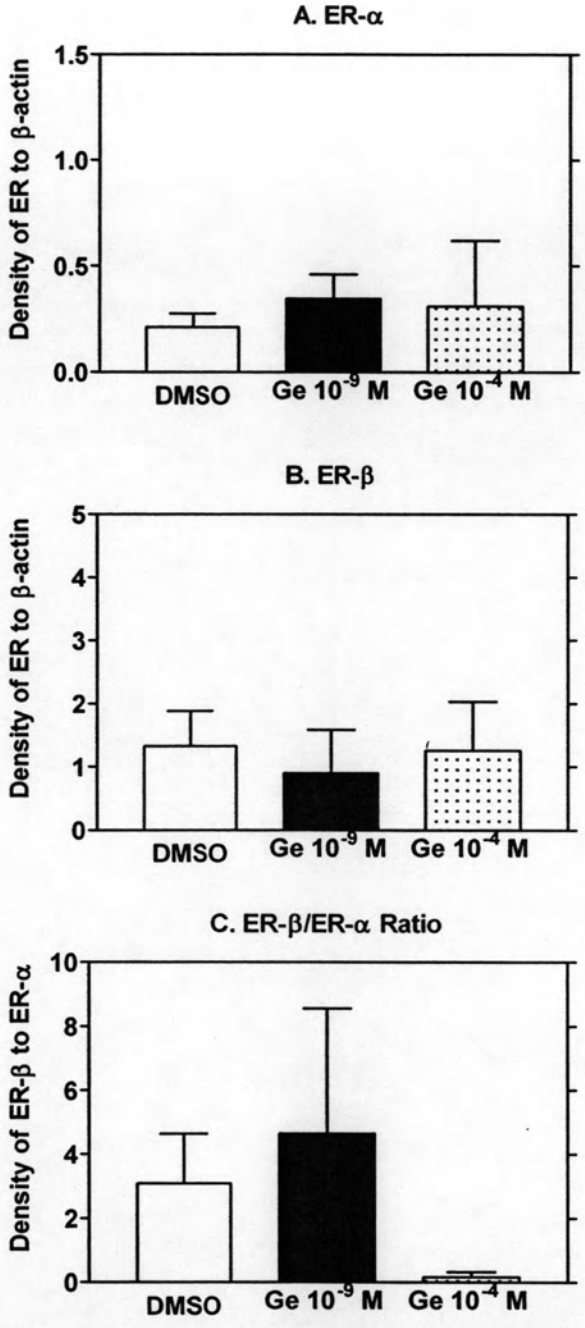

Figure 4-16 Histograms illustrate mean ± S.E.M of estrogen receptor protein levels of human endometrial cancer cells (RL-95) treated with genistein. A) ratio of ER-α/β-actin, B) ratio of ER-β/β-actin and C) ratio of ER-β/ER-α.

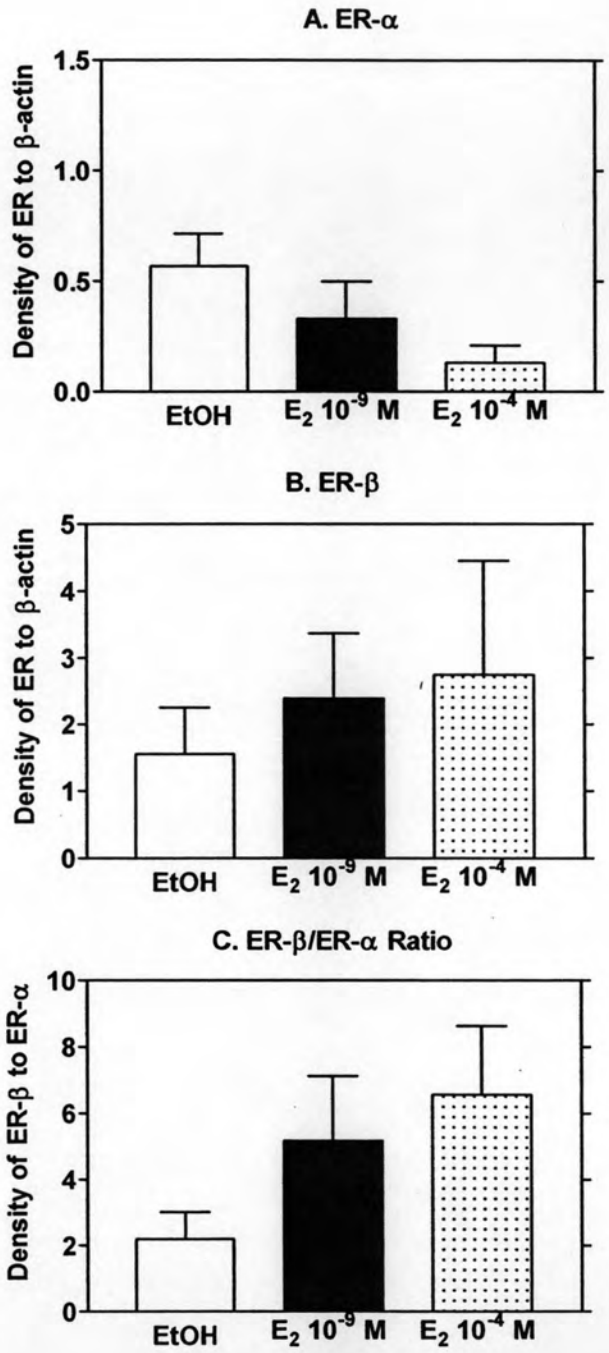

Figure 4-17 Histograms illustrate mean ± S.E.M of estrogen receptor protein levels of human endometrial cancer cells (RL-95) treated with 17β-estradiol. A) ratio of ER-α/β-actin, B) ratio of ER-β/β-actin and C) ratio of ER-β/ER-α.