CHAPTER IV
RESULTS

4.1 Physical factors

The two physical factors, amount of rain and air temperature in Ratchaburi province were monthly recorded by the Meteorological Department (Figure 4.1, 4.2). In this study we had described the period of study according to the amount of rain and air temperature into 3 seasons as follows; summer season, (from February to April) , rainy season, (from May to October) and winter season, (from November to January).

4.1.1 Monthly rain

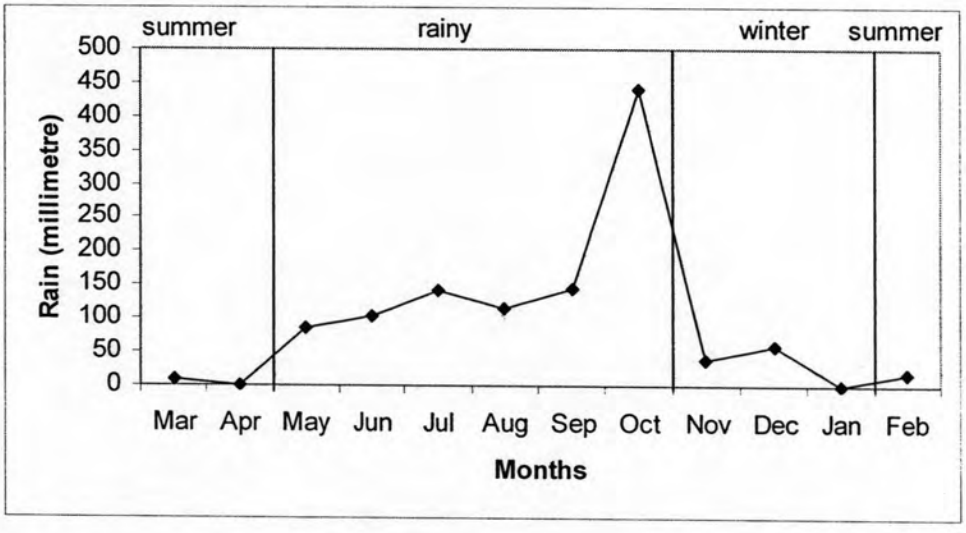


Figure 4.1 The amount of rain in Ratchaburi province since March 2005 to February 2006 recorded by the Meteorological Department.

4.1.2 Monthly temperature

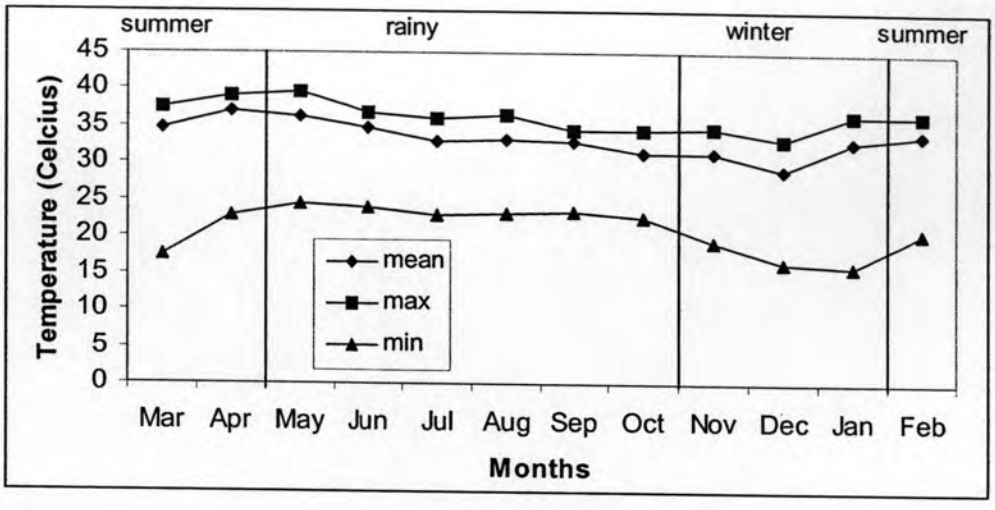


Figure 4.2 The temperature in Ratchaburi province since March 2005 to February 2006 recorded by the Meteorological Department.

4.2 Effects of *P. mirifica* on vaginal cornification in ovariectomized rats.

After 2 complete weeks of ovariectomy, the rats exhibited only leucocyted cells (L-type). It was confirmed by the completely disappearance of ovaries and no endrogenous ovarian estrogens was produced.

4.2.1 Control groups.

Negative control; the administration of distilled water did not influence the vaginal epithelium differentiation, only L-type cells were found (Figure 4.3).

Positive control; the daily subcutaneous injection of 200 μg/100g BW/day of 17β- estradiol for 14 days during the treatment period induced cornification of the vaginal epithelium cells as early as the next day of the treatment (D_2), and kept the Co-type cells until 6 days after the cessation of 17β- estradiol treatment (Figure 4.4).

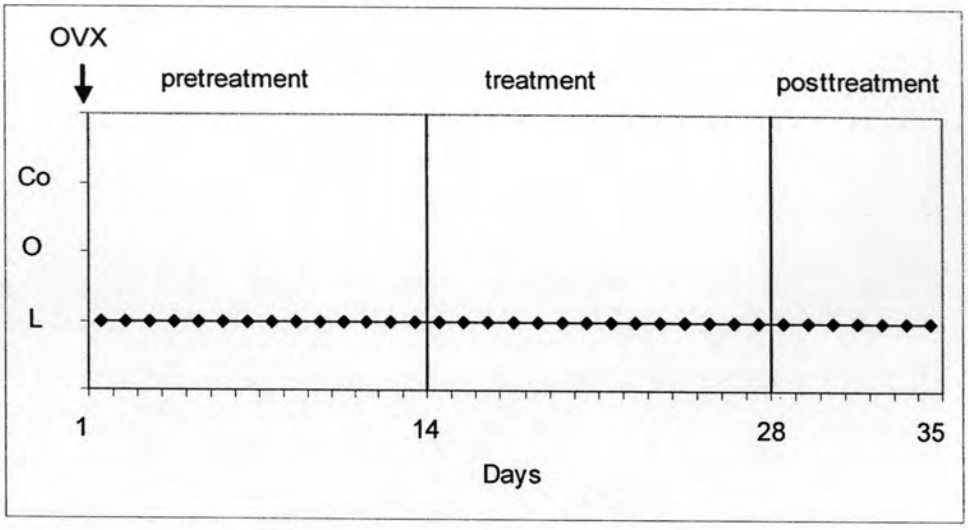


Figure 4.3 Differentiation of vaginal epithelium cells in rats treated with distilled water (Co cornified cells, O= nucleated cells, L = leucocyte cells, OVX= ovariectomy).

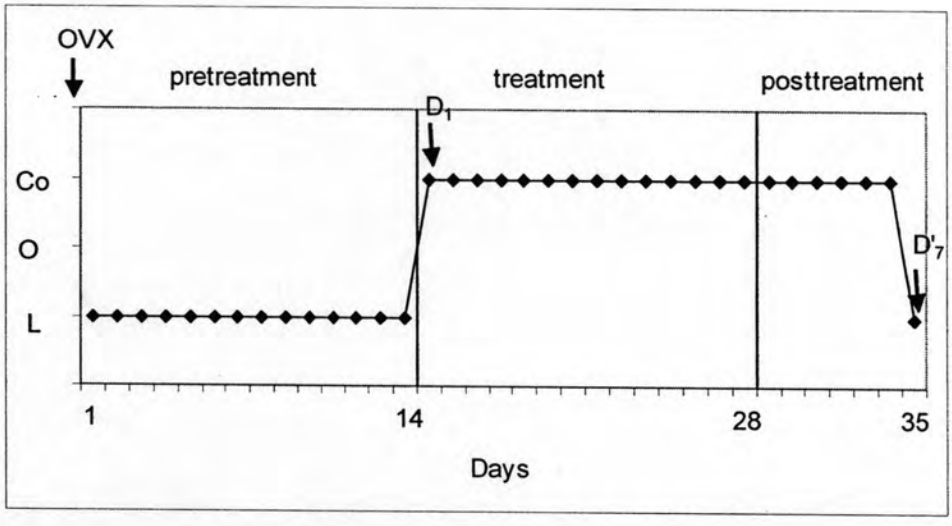


Figure 4.4 Differentiation of vaginal epithelium cells in rats treated with 200 μg/kg/day of 17β- estradiol (Co= cornified cells, O= nucleated cells, L= leucocyte cells, D=Day with appearance of cornified cells, D'= Day with appearance of leucocyte cells, OVX= ovariectomy).

4.2.1 *P. mirifica* treatment groups

The results are presented in Figure 4.5 to 4.16. It was described in term of collected seasons as follows;

Rainy season

The study of plant sample collected in May

Rats treated with **PM-III** sample at the dosage of **100 mg/kg BW/day**, the cell type was changed from L to Co within 9 days of the 14-day treatment period and reversed to L-type cells within 4 days after cessation of the treatment. Rats treated with **PM-IV** sample at the same dosage, the cell type was changed from L to Co within 9 days of the 14-day treatment period and reversed to L-type cells within 4 days after cessation of the treatment. Rats treated with **PM-III** sample at the dosage **1,000 mg/kg BW/day**, the cell type was changed from L-type cells to Co-type cell within 3 days of the 14-day treatment period and reversed to L-type cells within 4 days after cessation of the treatment. Rats treated with **PM-IV** sample at the same dosage, the cell type was changed from L-type cells to Co-type cells within 2 days of the 14-day treatment period and recovered within 6 days after cessation of the treatment (Figure 4.5).

The study of plant sample collected in June

Rats treated with **PM-III** sample at the dosage of **100 mg/kg BW/day**, the cell type was changed from L to Co within 8 days of the 14-day treatment period and reversed to L-type cells within 4 days after cessation of the treatment. Rats treated with **PM-IV** sample at the same dosage, the cell type was changed from L to Co within 8 days of the 14-day treatment period and reversed to L-type cells within 4 days after cessation of the treatment. Rats treated with **PM-III** sample at the dosage of **1,000 mg/kg BW/day**, the cell type was changed from L-type cells to Co-type cells within 3 days of the 14-day treatment period and reversed to L-type cells within 5 days after cessation of the treatment. Rats treated with **PM-IV** sample at the same dosage, the cell type was changed from L-type cells to Co-type cells within 2 days of the 14-day treatment period and recovered within 5 days after cessation of the treatment (Figure 4.6).

The study of plant sample collected in July

Rats treated with **PM-III** sample at the dosage of **100 mg/kg BW/day** did not have any effect on vaginal epithelium, only L-type cells was found. Rats treated with **PM-IV** sample at the same dosage did not have any effect on vaginal epithelium, only L-type cells were found, similarly. Rats treated with **PM-III** sample at the dosage of **1,000 mg/kg BW/day**, the cell type was changed from L-type cells to Co-type cells within 4 days of the 14-day treatment period and reversed to L-type cells within 5 days after cessation of the treatment. Rats treated with **PM-IV** sample at the same dosage, the cell type was changed from L-type cells to Co-type cells within 3 days of the 14-day treatment period and recovered within 6 days after cessation of the treatment (Figure 4.7).

The study of plant sample collected in August

Rats treated with **PM-III** sample at the dosage of **100 mg/kg BW/day** did not have any effect on vaginal epithelium, only L-type cells was found. Rats treated with **PM-IV** sample at the same dosage did not have any effect on vaginal epithelium, only L-type cells were found, similarly. Rats treated with **PM-III** sample at the dosage of **1,000 mg/kg BW/day**, the cell type was changed from L-type cells to Co-type cells within 3 days of the 14-day treatment period and reversed to L-type cells within 5 days after cessation of the treatment. Rats treated with **PM-IV** sample at the same dosage, the cell type was changed from L-type cells to Co-type cells within 2 days of the 14-day treatment period and recovered within 6 days after cessation of the treatment (Figure 4.8).

The study of plant sample collected in September

Rats treated with **PM-III** sample at the dosage of **100 mg/kg BW/day** did not have any effect on vaginal epithelium, only L-type cells was found. Rats treated with **PM-IV** sample at the same dosage did not have any effect on vaginal epithelium, only L- type cells was found, similarly. Rats treated with **PM-III** sample at the dosage of **1,000 mg/kg BW/day**, the cell type was changed from L-type cells to Co-type cells within 4 days of the 14-day treatment period and reversed to L-type cells within 4 days after cessation of the treatment. Rats treated with **PM-IV** sample at the same dosage, the cell type was changed from L-type cells to Co-type cells within 2 days of the 14-day treatment period and recovered within 5 days after cessation of the treatment (Figure 4.9).

The study of plant sample collected in October

Rats treated with **PM-III** sample at the dosage of **100 mg/kg BW/day** did not have any effect on vaginal epithelium, only L-type cells was found. Rats treated with **PM-IV** sample at the same dosage did not have any effect on vaginal epithelium, only L-type cells were found, similarly. Rats treated with **PM-III** sample at the dosage of **1,000 mg/kg BW/day**, the cell type was changed from L-type cells to Co-type cells within 4 days of the 14-day treatment period and reversed to L-type cells within 5 days after cessation of the treatment. Rats treated with **PM-IV** sample at the same dosage, the cell type was changed from L-type cells to Co-type cells within 3 days of the 14-day treatment period and recovered within 5 days after cessation of the treatment (Figure 4.10).

Winter season

The study of plant sample collected in November

Rats treated with **PM-III** sample at the dosage of **100 mg/kg BW/day**, the cell type was changed from L to Co within 6 days of the 14-day treatment period and reversed to L-type cells within 4 days after cessation of the treatment. Rats treated with **PM-IV** sample at the same dosage, the cell type was changed from L to Co within 6 days of the 14-day treatment period and reversed to L-type cells within 5 days after cessation of the treatment. Rats treated with **PM-III** sample at the dosage of **1,000 mg/kg BW/day**, the cell type was changed from L-type cells to Co-type cells within 2 days of the 14-day treatment period and reversed to L-type cells within 5 days after cessation of the treatment. Rats treated with **PM-IV** sample at the same dosage, the cell type was changed from L-type cells to Co-type cells within 2 days of the 14-day treatment period and recovered within 6 days after cessation of the treatment (Figure 4.11).

The study of plant sample collected in December

Rats treated with **PM-III** sample at the dosage of **100 mg/kg BW/day**, the cell type was changed from L to Co within 6 days of the 14-day treatment period and reversed to L-type cells within 4 days after cessation of the treatment. Rats treated with **PM-IV** sample at the same dosage, the cell type was changed from L to Co within 6 days of the 14-day treatment period and reversed to L-type cells within 4 days after cessation of the treatment. Rats treated with **PM-III** sample at the dosage of **1,000 mg/kg BW/day**, the cell type was changed from L-type cells to Co-

type cells within 3 days of the 14-day treatment period and reversed to L-type cells within 3 days after cessation of the treatment. Rats treated with **PM-IV** sample at the same dosage, the cell type was changed from L-type cells to Co-type cells within 2 days of the 14-day treatment period and recovered within 5 days after cessation of the treatment (Figure 4.12).

The study of plant sample collected in January

Rats treated with **PM-III** sample at the dosage of **100 mg/kg BW/day**, the cell type was changed from L to Co within 6 days of the 14-day treatment period and reversed to L-type cells within 4 days after cessation of the treatment. Rats treated with **PM-IV** sample at the same dosage, the cell type was changed from L to Co within 5 days of the 14-day treatment period and reversed to L-type cells within 6 days after cessation of the treatment. Rats treated with **PM-III** sample at the dosage of **1,000 mg/kg BW/day**, the cell type was changed from L-type cells to Co-type cells within 2 days of the 14-day treatment period and reversed to L-type cells within 6 days after cessation of the treatment. Rats treated with **PM-IV** sample at the same dosage, the cell type was changed from L-type cells to Co-type cells within 2 days of the 14-day treatment period and recovered within 6 days after cessation of the treatment (Figure 4.13).

Summer season

The study of plant sample collected in February

Rats treated with **PM-III** sample at the dosage of **100 mg/kg BW/day**, the cell type was changed from L to Co within 7 days of the 14-day treatment period and reversed to L-type cells within 4 days after cessation of the treatment. Rats treated with **PM-IV** sample at the same dosage, the cell type was changed from L to Co within 6 days of the 14-day treatment period and reversed to L-type cells within 4 days after cessation of the treatment. Rats treated with **PM-III** sample at the dosage of **1,000 mg/kg BW/day**, the cell type was changed from L-type cells to Co-type cells within 3 days of the 14-day treatment period and reversed to L-type cells within 6 days after cessation of the treatment. Rats treated with **PM-IV** sample at the same dosage, the cell type was changed from L-type cells to Co-type cells within 2 days of the 14-day treatment period and recovered within 6 days after cessation of the treatment (Figure 4.14).

The study of plant sample collected in March

Rats treated with **PM-III** sample at the dosage of **100 mg/kg BW/day**, the cell type was changed from L to Co within 7 days of the 14-day treatment period and reversed to L-type cells within 4 days after cessation of the treatment. Rats treated with **PM-IV** sample at the same dosage, the cell type was changed from L to Co within 6 days of the 14-day treatment period and reversed to L-type cells within 4 days after cessation of the treatment. Rats treated with **PM-III** sample at the dosage of **1,000 mg/kg BW/day**, the cell type was changed from L-type cells to Co-type cells within 3 days of the 14-day treatment period and reversed to L-type cells within 5 days after cessation of the treatment. Rats treated with **PM-IV** sample at the same dosage, the cell type was changed from L-type cells to Co-type cells within 2 days of the 14-day treatment period and recovered within 6 days after cessation of the treatment (Figure 4.15).

The study of plant sample collected in April

Rats treated with **PM-III** sample at the dosage of **100 mg/kg BW/day**, the cell type was changed from L to Co within 6 days of the 14-day treatment period and reversed to L-type cells within 4 days after cessation of the treatment. Rats treated with **PM-IV** sample at the same dosage, the cell type was changed from L to Co within 6 days of the 14-day treatment period and reversed to L-type cells within 5 days after cessation of the treatment. Rats treated with **PM-III** sample at the dosage of **1,000 mg/kg BW/day**, the cell type was changed from L-type cells to Co-type cells within 3 days of the 14-day treatment period and reversed to L-type cells within 5 days after cessation of the treatment. Rats treated with **PM-IV** sample at the same dosage, the cell type was changed from L-type cells to Co-type cells within 2 days of the 14-day treatment period and recovered within 6 days after cessation of the treatment (Figure 4.16).

Rainy season

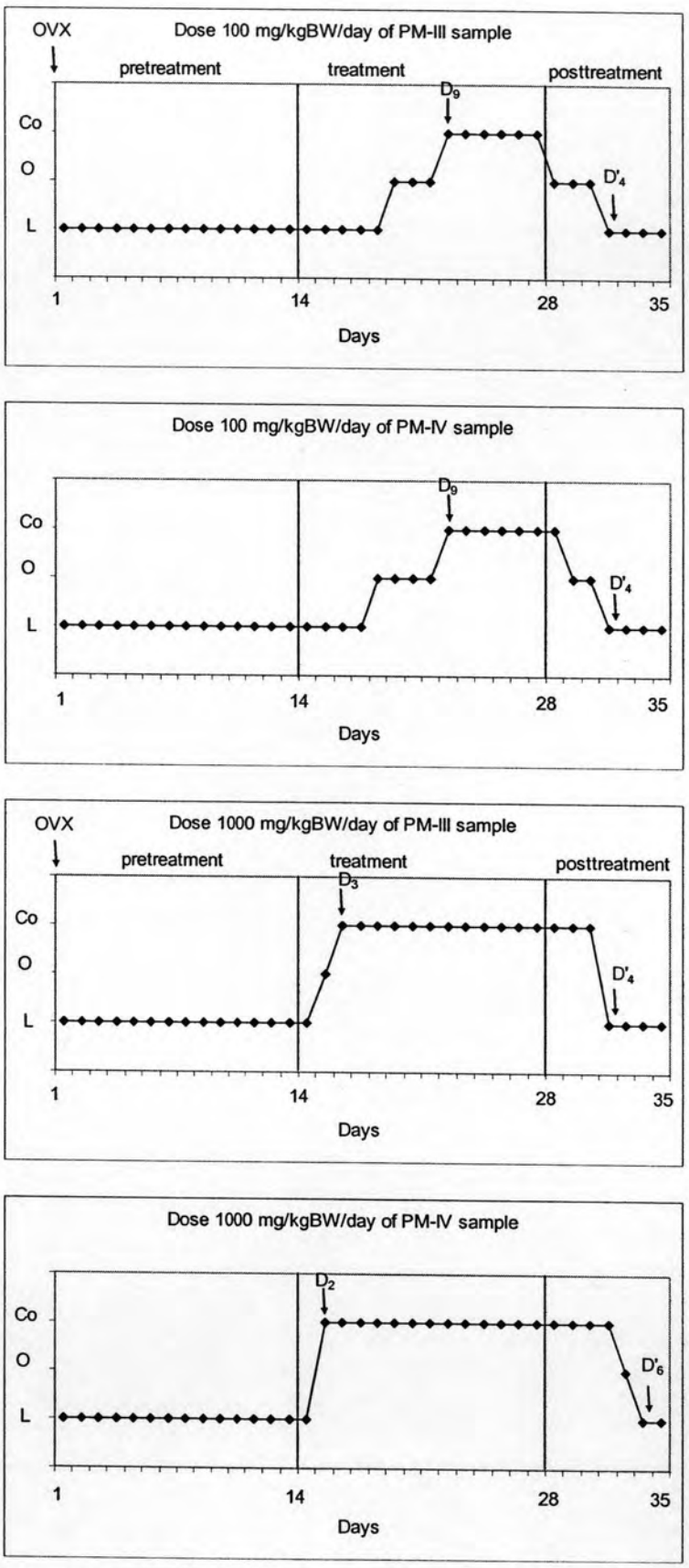


Figure 4.5 Changes of vaginal epithelium cells in rats treated with 100 and 1,000 mg/kgBW/day of PM-III and PM-IV powder derived from tuber collected in **May** (Co = cornified cells, O = nucleated cells, L = leucocyte cells, D = Day with appearance of cornified cells, D = Day with appearance of leucocyte cells)

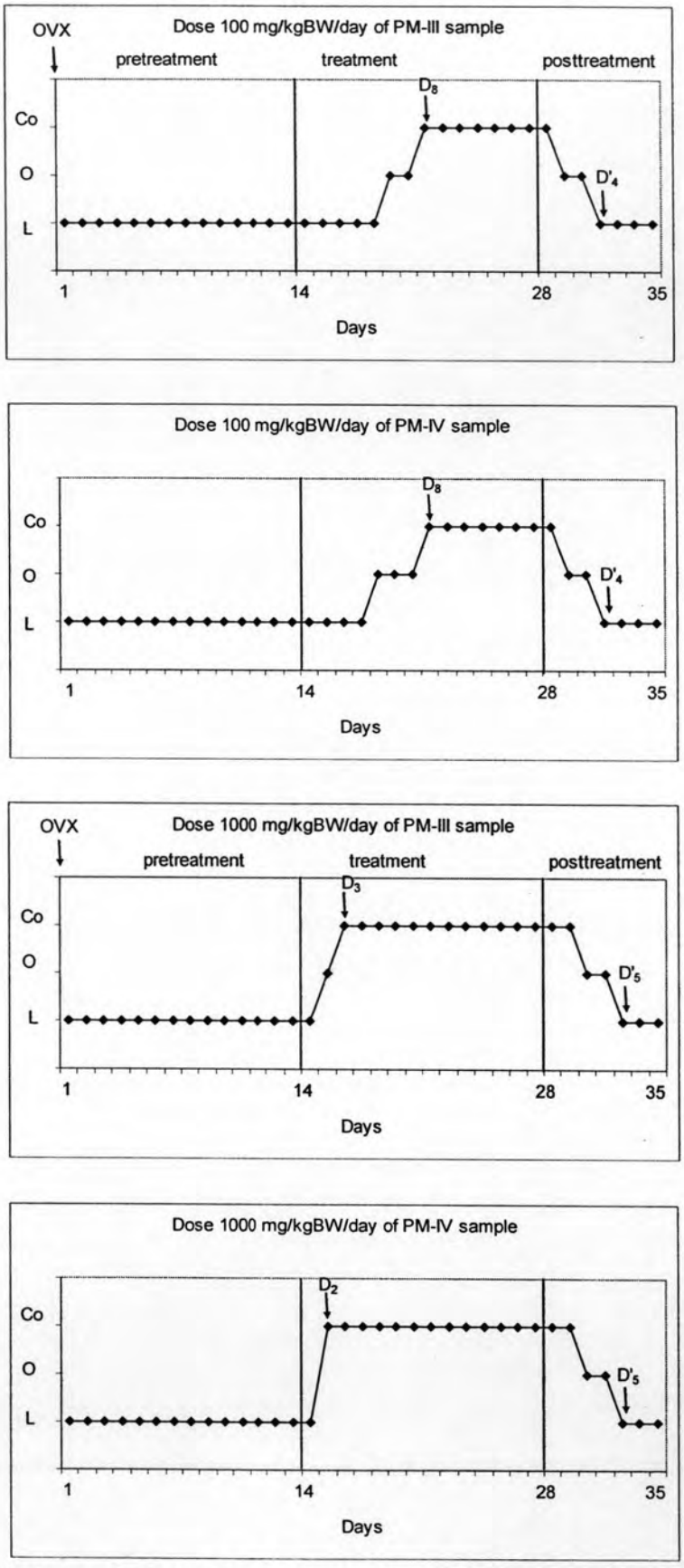


Figure 4.6 Changes of vaginal epithelium cells in rats treated with 100 and 1,000 mg/kgBW/day of PM-III and PM-IV powder derived from tuber collected in **June** (Co = cornified cells, O = nucleated cells, L = leucocyte cells, D = Day with appearance of cornified cells, D = Day with appearance of leucocyte cells)

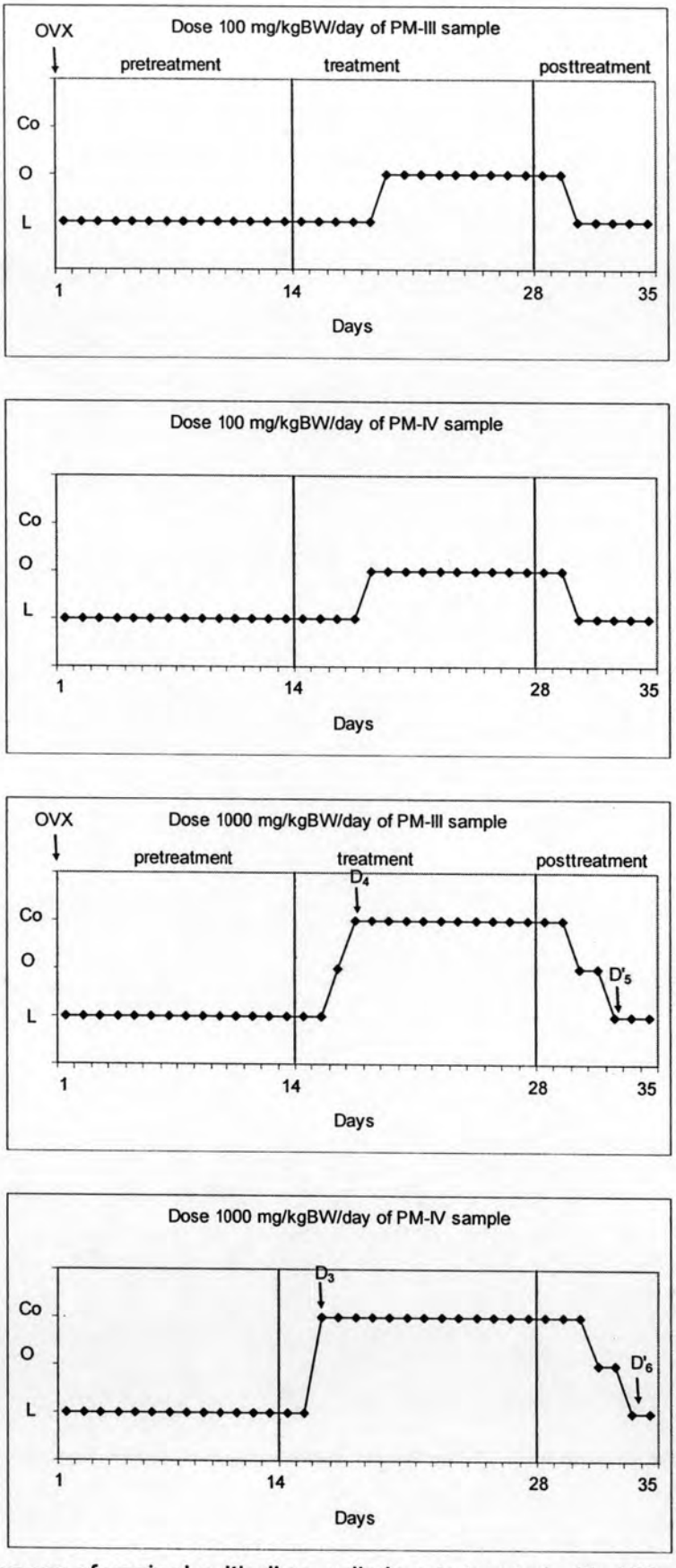


Figure 4.7 Changes of vaginal epithelium cells in rats treated with 100 and 1,000 mg/kgBW/day of PM-III and PM-IV powder derived from tuber collected in **July** (Co = cornified cells, O = nucleated cells, L = leucocyte cells, D = Day with appearance of cornified cells, D = Day with appearance of leucocyte cells)

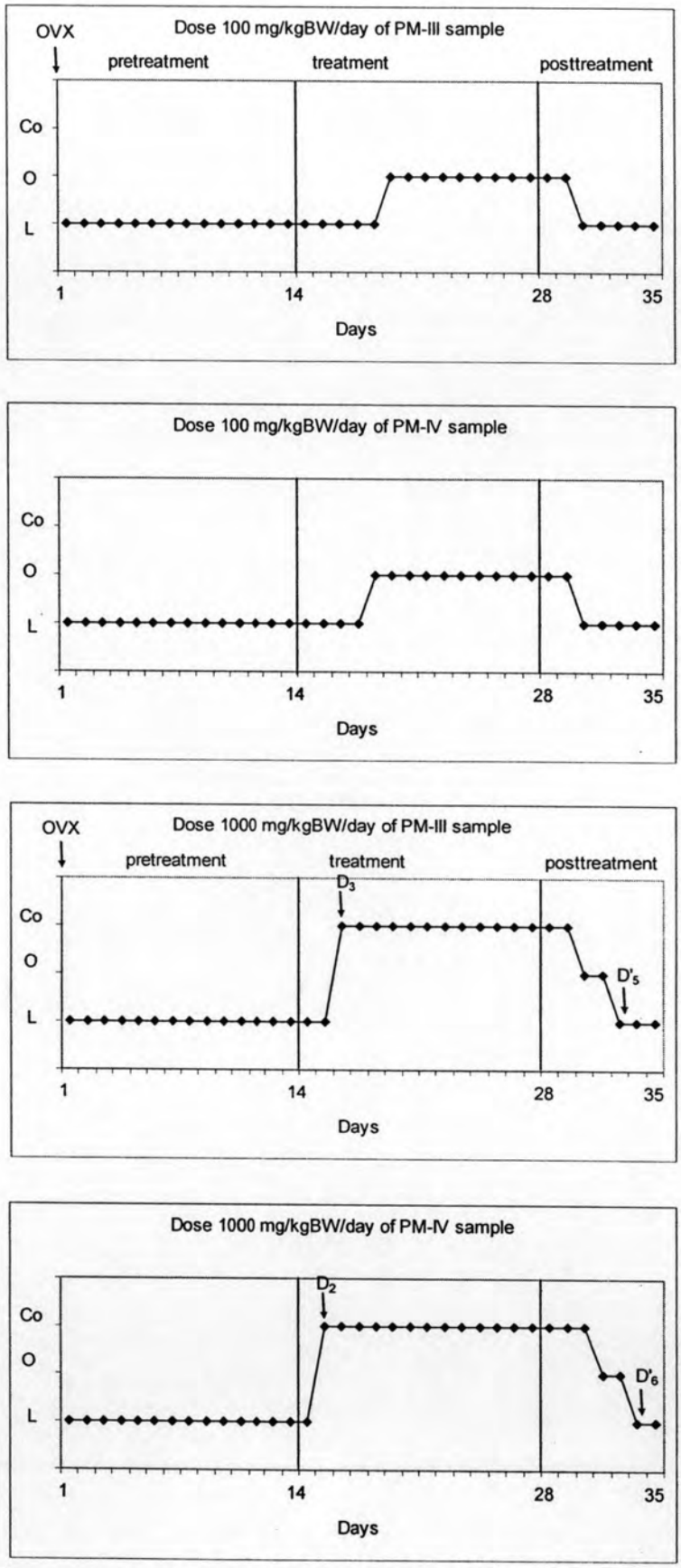


Figure 4.8 Changes of vaginal epithelium cells in rats treated with 100 and 1,000 mg/kgBW/day of PM-III and PM-IV powder derived from tuber collected in **August** (Co = cornified cells, O = nucleated cells, L = leucocyte cells, D = Day with appearance of cornified cells, D = Day with appearance of leucocyte cells)

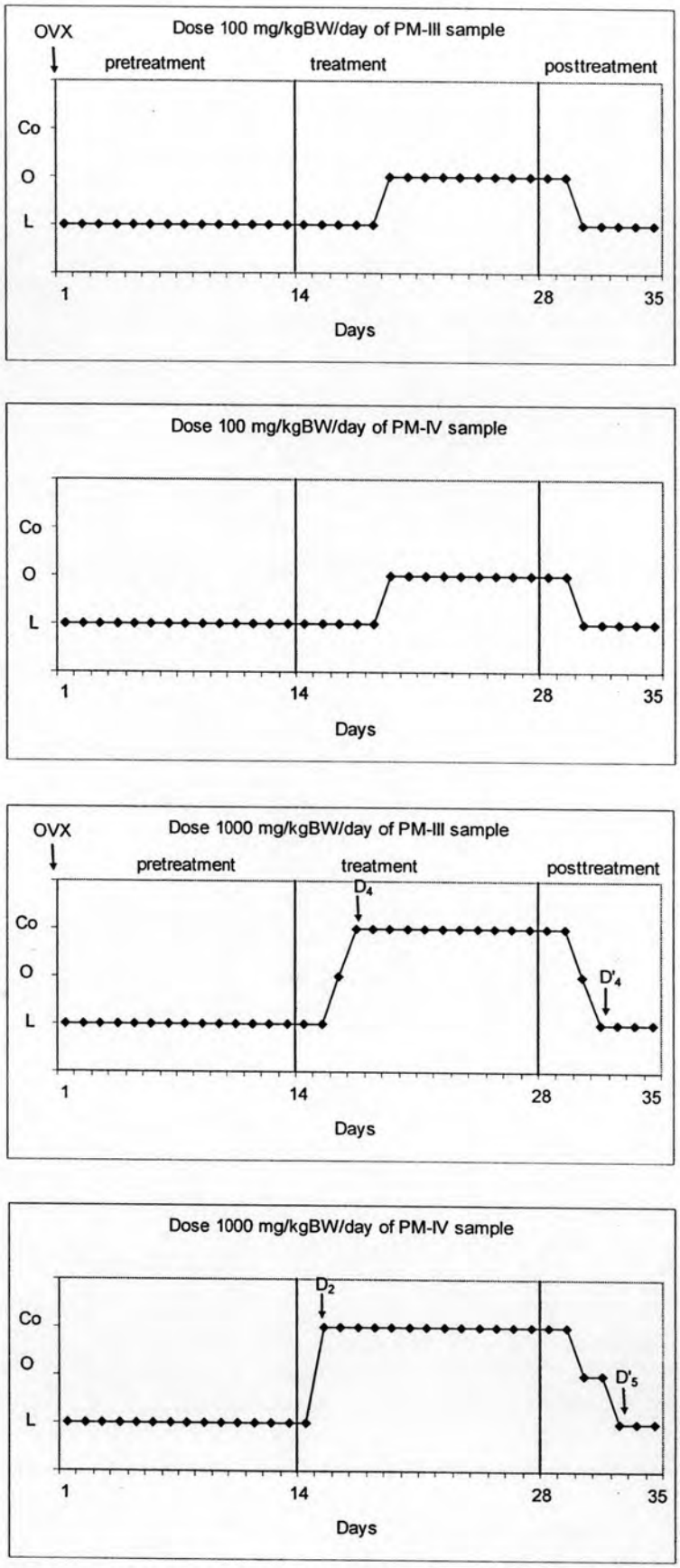


Figure 4.9 Changes of vaginal epithelium cells in rats treated with 100 and 1,000 mg/kgBW/day of PM-III and PM-IV powder derived from tuber collected in **September** (Co = cornified cells, O = nucleated cells, L = leucocyte cells, D = Day with appearance of cornified cells, D = Day with appearance of leucocyte cells)

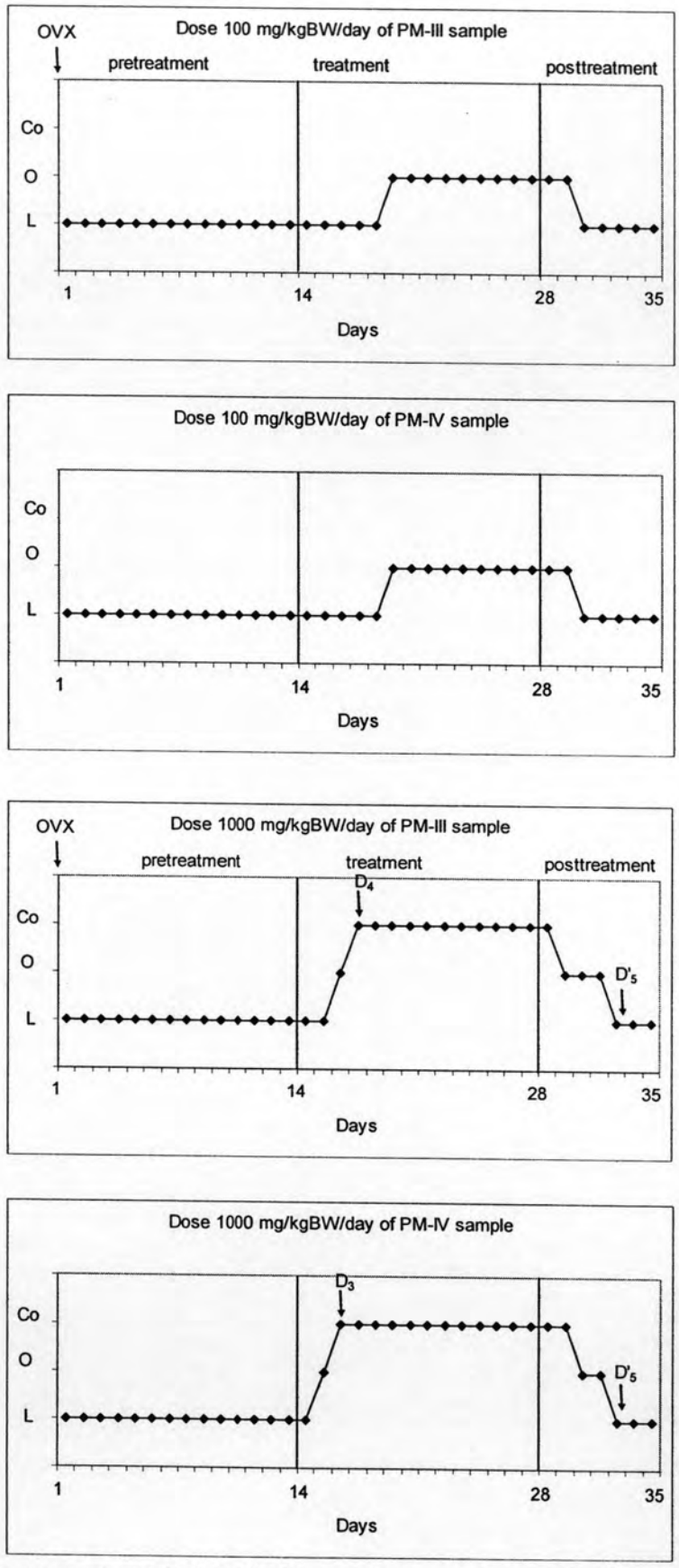


Figure 4.10 Changes of vaginal epithelium cells in rats treated with 100 and 1,000 mg/kgBW/day of PM-III and PM-IV powder derived from tuber collected in **October** (Co = cornified cells, O = nucleated cells, L = leucocyte cells, D = Day with appearance of cornified cells, D = Day with appearance of leucocyte cells)

Winter season

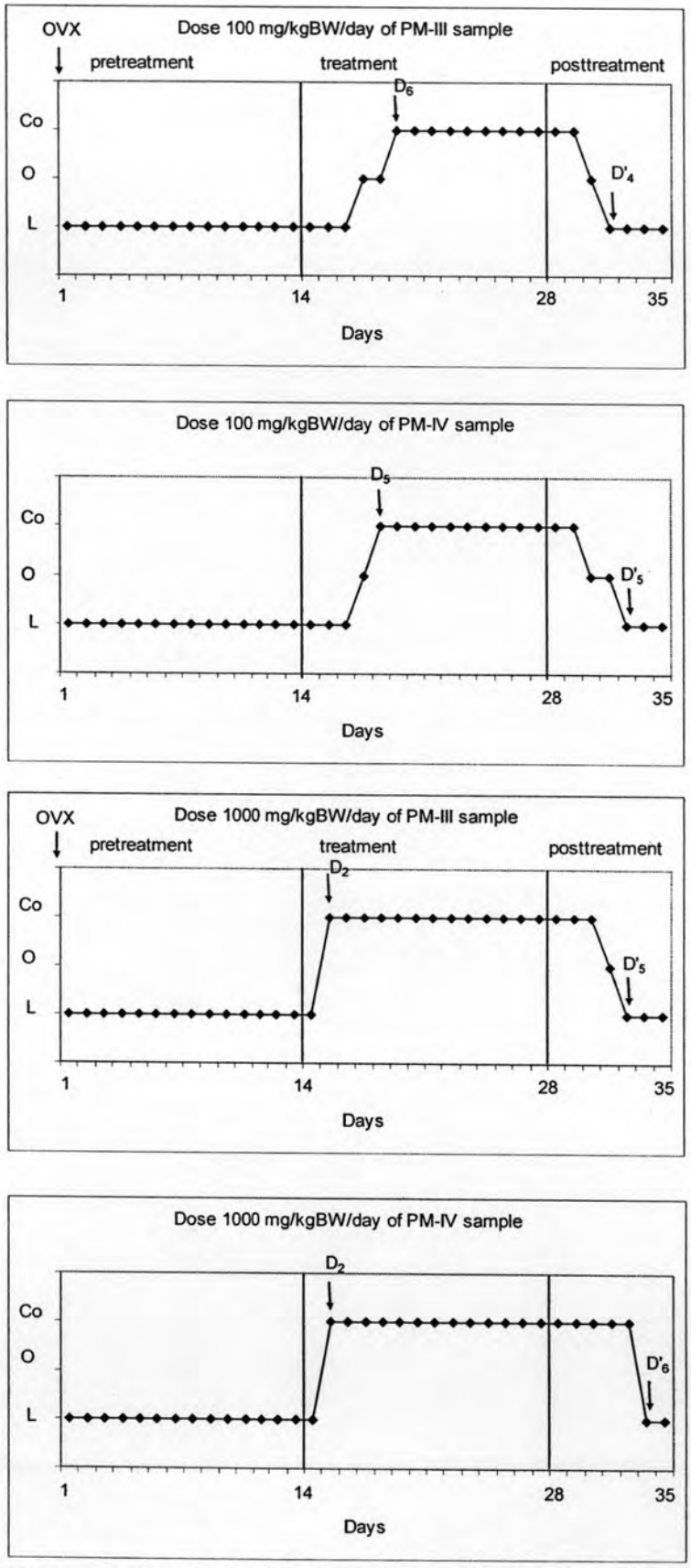


Figure 4.11 Changes of vaginal epithelium cells in rats treated with 100 and 1,000 mg/kgBW/day of PM-III and PM-IV powder derived from tuber collected in **November** (Co = cornified cells, O = nucleated cells, L = leucocyte cells, D = Day with appearance of cornified cells, D = Day with appearance of leucocyte cells)

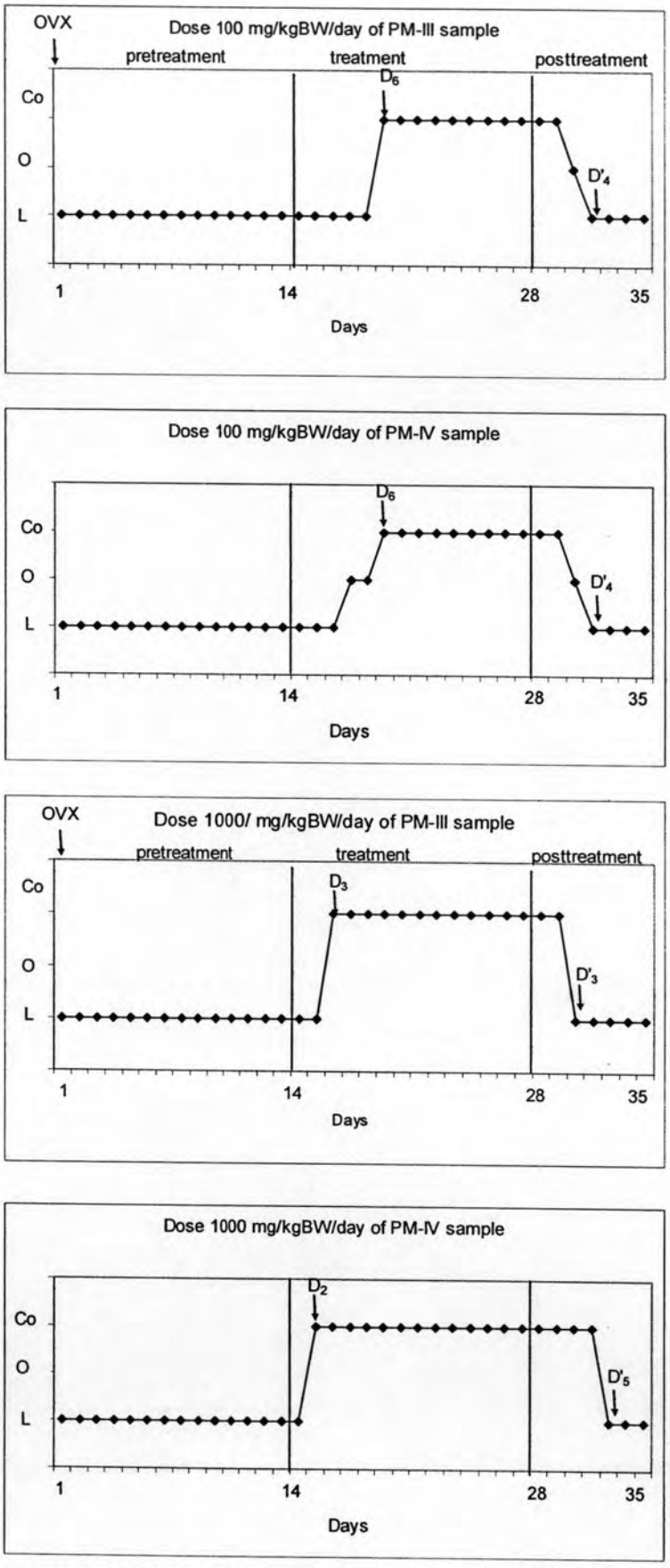


Figure 4.12 Changes of vaginal epithelium cells in rats treated with 100 and 1,000 mg/kgBW/day of PM-III and PM-IV powder derived from tuber collected in **December** (Co = cornified cells, O = nucleated cells, L = leucocyte cells, D = Day with appearance of cornified cells, D = Day with appearance of leucocyte cells)

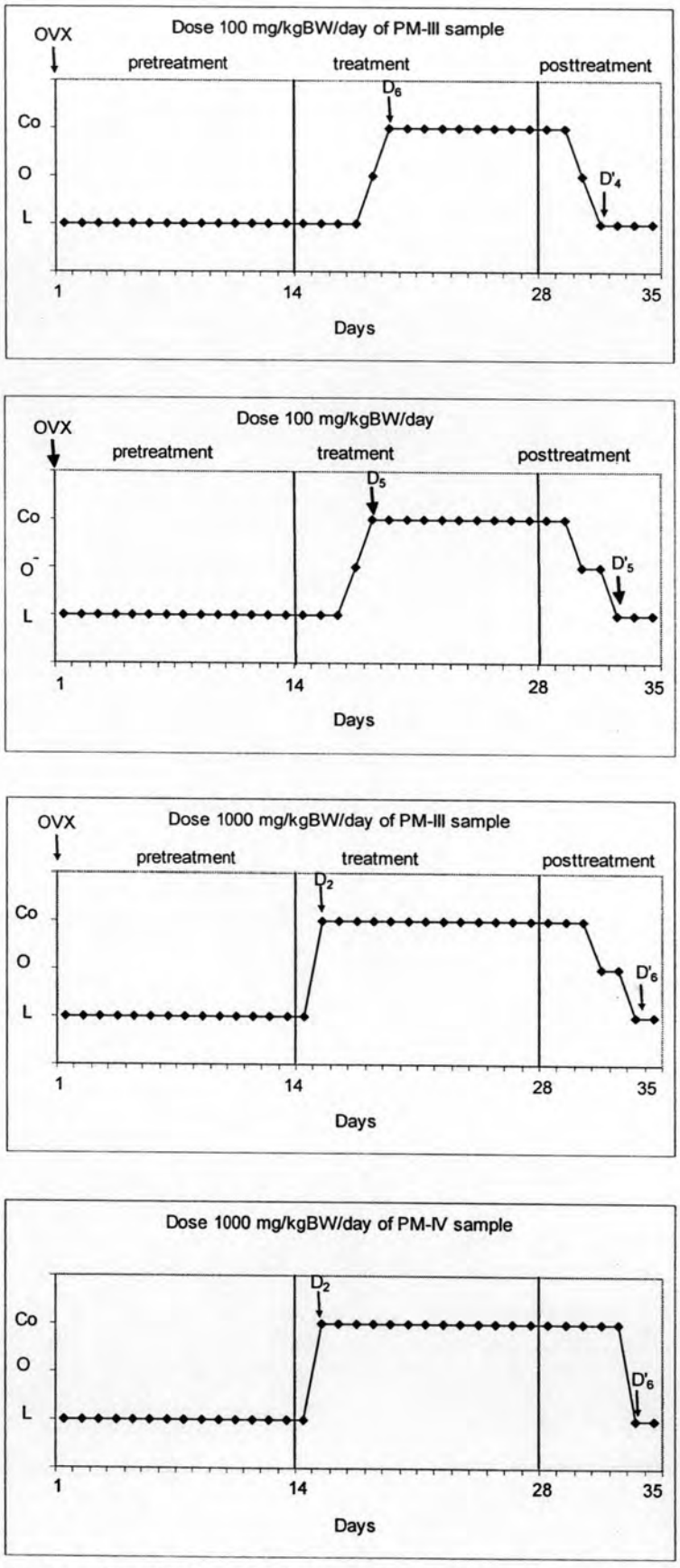


Figure 4.13 Changes of vaginal epithelium cells in rats treated with 100 and 1,000 mg/kgBW/day of PM-III and PM-IV powder derived from tuber collected in **January** (Co = cornified cells, O = nucleated cells, L = leucocyte cells, D = Day with appearance of cornified cells, D = Day with appearance of leucocyte cells)

Summer season

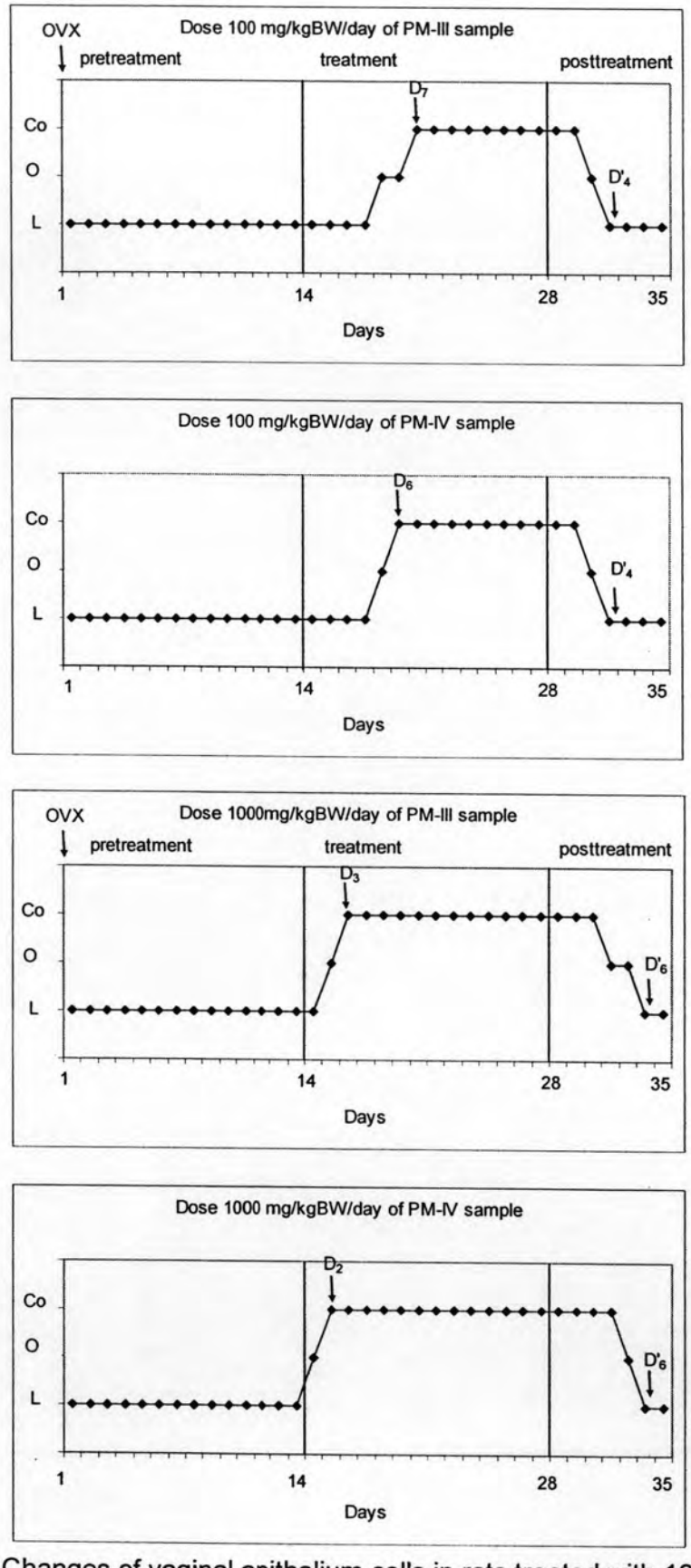
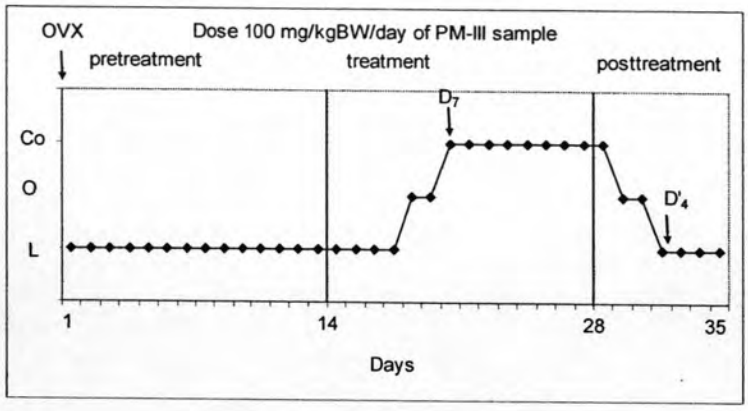
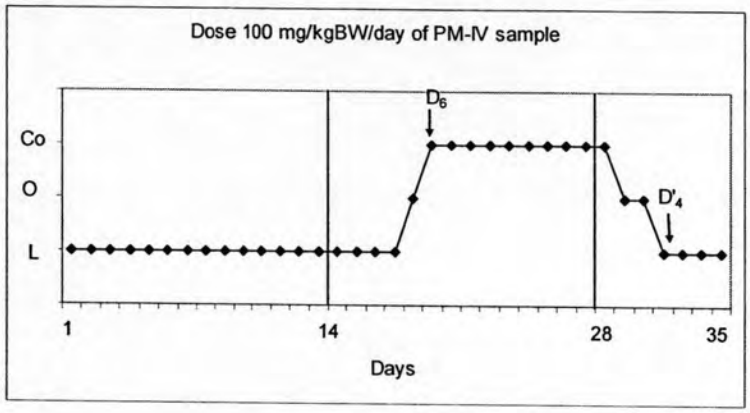
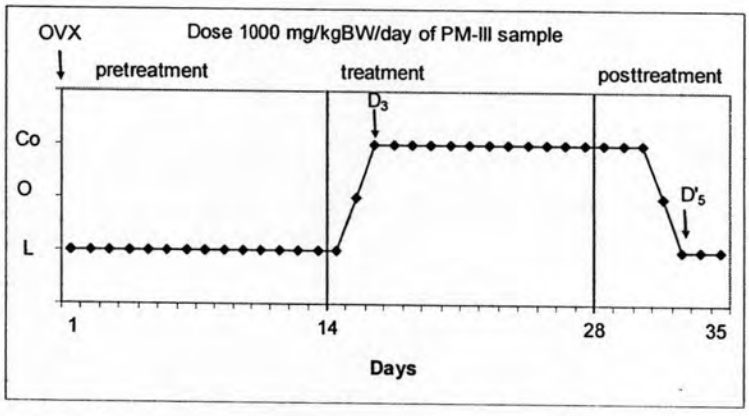


Figure 4.14 Changes of vaginal epithelium cells in rats treated with 100 and 1,000 mg/kgBW/day of PM-III and PM-IV powder derived from tuber collected in **February** (Co = cornified cells, O = nucleated cells, L = leucocyte cells, D = Day with appearance of cornified cells, D = Day with appearance of leucocyte cells)







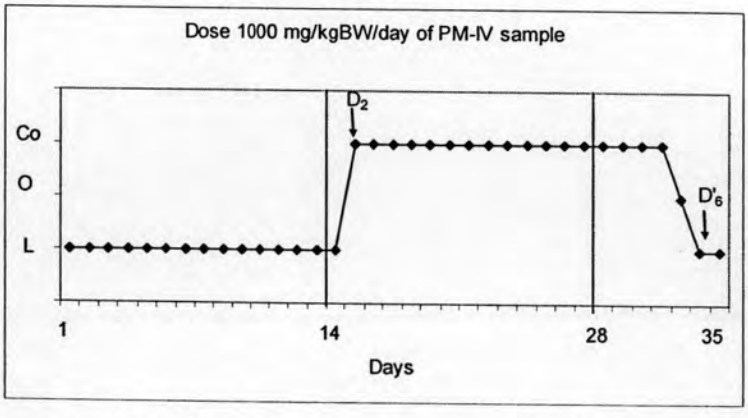


Figure 4.15 Changes of vaginal epithelium cells in rats treated with 100 and 1,000 mg/kgBW/day of PM-III and PM-IV powder derived from tuber collected in **March** (Co = cornified cells, O = nucleated cells, L = leucocyte cells, D = Day with appearance of cornified cells, D = Day with appearance of leucocyte cells)

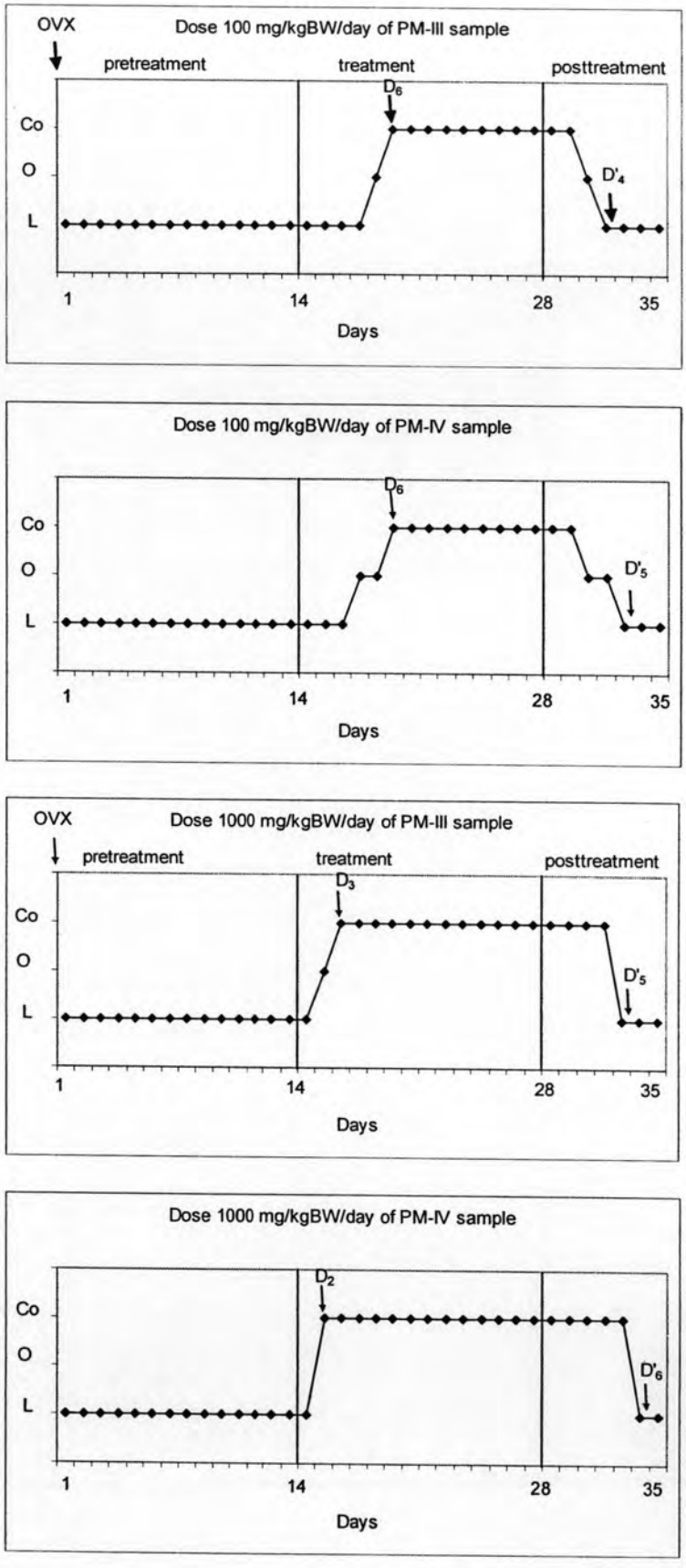


Figure 4.16 Changes of vaginal epithelium cells in rats treated with 100 and 1,000 mg/kgBW/day of PM-III and PM-IV powder derived from tuber collected in **April** (Co = cornified cells, O = nucleated cells, L = leucocyte cells, D = Day with appearance of cornified cells, D = Day with appearance of leucocyte cells)

From the changes of vaginal epithelium cells in rats treated with 100 and 1,000 mg/kg BW/day of *P. mirifica* powder (PM-III and PM-IV), there are differences in the first day of appearance of cornified cells during treatment period. It could be summarized in Table 4.1. The appearances of leucocyte cells during post-treatment were also summarized in Table 4.2.

Table 4.1 First day of appearance of cornified cells in rats after treated with 2 cultivars of *Pueraria mifica* (PM-III and PM-IV) collected in 12 months, distilled water and 17β-estradiol, during treatment period (N^L = No cornified cells, and leucocyte cells were found throughout the experiment period and 14 days were used as a number for statistical analysis).

season	month	PM-III		PM-IV	
		dose 100 mg/kg BW/day	dose 1,000 mg/kg BW/day	dose 100 mg/kg BW/day	dose 1,000 mg/kg BW/day
Rainy	May	9	3	9	2
	June	8	3	8	2
	July	N^L	4	N^L	3
	August	N^L	3	N^L	2
	September	N^L	4	N^L	2
	October	N^L	4	N^L	3
mean ± S.E.M. (n=6)		12.17 ± 1.17*	3.50 ± 0.22 *	12.17 ± 1.17*	2.33 ± 0.21^{ns}
Winter	November	6	2	5	2
	December	6	3	6	2
	January	6	2	5	2
mean ± S.E.M. (n=3)		6.00 ± 0.00	2.33 ± 0.33	5.33 ± 0.33	2.00 ± 0.00^{ns}
Summer	February	7	3	6	2
	March	7	3	6	2
	April	6	3	6	2
mean ± S.E.M. (n=3)		6.67 ± 0.33	3.00 ± 0.00	6.00 ± 0.00	2.00 ± 0.00^{ns}
Control groups	DW	N^L			
	E2	1			

* ;significant difference among rainy, winter and summer

^{ns} ;significant difference among rainy, winter and summer

From the statistical analysis of the period (day) of appearance of cornified cells among 3 seasons; rainy, winter and summer in Table 4.1 it was found that *P. mirifica* collected in July, August, September and October did not show vaginal epithelium cornification at the dosage of 100 mg/kg BW/day. Samples collected in rainy season were statistical differences among those 3 seasons. The dosage of 100 and 1,000 mg/kg BW/day of PM-III and PM-IV collected in winter season tended to exhibit higher estrogenic activity (sooner of first day of appearance of cornified cells) than summer and rainy seasons, respectively.

Table 4.2 First day of appearance of leucocyte cells in rats after treated with 2 cultivars of *Pueraria mifica* (PM-III and PM-IV) collected during 12 months, distilled water and 17β-estradiol during post-treatment period (N^L = No cornified cells, and leucocyte cells were found throughout the experiment period).

season	month	PM-III		PM-IV	
		dose 100 mg/kgBW/day	dose 1,000 mg/kgBW/day	dose 100 mg/kgBW/day	dose 1,000 mg/kgBW/day
Rainy	May	4	4	4	6
	June	4	5	4	5
	July	3	5	3	6
	August	3	5	3	6
	September	3	4	3	5
	October	3	5	3	5
mean ± S.E.M. (n=6)		3.33 ± 0.21^{ns}	4.67 ± 0.21^{ns}	3.33 ± 0.21*	5.50 ± 0.22^{ns}
Winter	November	4	5	5	6
	December	2	3	4	5
	January	4	6	5	6
mean ± S.E.M. (n=3)		3.33 ± 0.67^{ns}	4.67 ± 0.88^{ns}	4.67 ± 0.33	5.67 ± 0.33^{ns}
Summer	February	4	6	4	6
	March	4	5	4	6
	April	4	5	5	6
mean ± S.E.M. (n=3)		4.00 ± 0.00^{ns}	5.33 ± 0.33^{ns}	4.33 ± 0.33	6.00 ± 0.00^{ns}
Control groups	DW	1			
	E2	7			

* ;significant difference among rainy, winter and summer

ns ;significant difference among rainy, winter and summer

From the statistical analysis of the first day of appearance of leucocytes cells among 3 seasons; rainy, winter and summer in Table 4.2, it was found that there was no statistical difference in the dose of 100 and 1,000 mg/kg BW/day of PM-III and PM-IV. However, at the dose of 1000 mg/kg BW/day tended to exhibit stronger estrogenic activity (longer day of leucocyte apperrances) than the dosage 1,000 mg/kg BW/day. But there were statistical differences among 3 seasons in the dosage of 100 and 1,000 mg/kg BW/day also showed the trend of stronger estrogenic activity in PM-IV than PM-III.

When the data of Table 4.1 and 4.2 were taken into account and calculated for the duration of the appearance of cornified cells during the treatment and post-treatment periods, the estrogenic activity of PM-III and PM-IV collected in 12 months could be ranked as shown in Table 4.3 and Figure 17, 18. Samples collected in rainy season were statistical difference from the others. The dose of 100 and 1,000 mg/kg BW/day of PM-III and PM-IV collected in winter tended to show the highest estrogenic activity (the longest duration of appearances of cornified cells). In addition, PM-III and PM-IV collected in rainy season tended to show the lowest estrogenic activity (the shortest duration of appearances of cornified cells).

Comparison among 3 months in winter season, *P. mirifica* collected in November and January showed the highest estrogenic activity in both doses. Comparison among 6 months in rainy season, *P. mirifica* collected in October showed the lowest estrogenic activity in the dose of 1,000 mg/kg BW/day of PM-III and PM-IV.

Table 4.3 The duration of appearance of cornified cells during treatment and post-treatment periods.

season	month	PM-III		PM-IV	
		dose 100 mg/kgBW/day	dose 1,000 mg/kgBW/day	dose 100 mg/kgBW/day	dose 1,000 mg/kgBW/day
Rainy	May	6	15	7	17
	June	8	14	8	15
	July	0	13	0	16
	August	0	14	0	16
	September	0	13	0	15
	October	0	12	0	14
mean ± S.E.M. (n=6)		2.33 ± 1.50*	13.50 ± 0.43*	2.50 ± 1.59*	15.50 ± 0.43*
Winter	November	11	16	12	18
	December	11	14	11	17
	January	11	16	12	18
mean ± S.E.M. (n=3)		11.00 ± 0.00	15.33 ± 0.67	11.67 ± 0.33	17.67 ± 0.33
Summer	February	10	15	11	17
	March	9	15	10	17
	April	11	16	11	18
mean ± S.E.M. (n=3)		10.00 ± 0.58	15.33 ± 0.33	10.67 ± 0.33	17.33 ± 0.33
Control groups	DW	0.00 ± 0.00			
	E2	20.00 ± 0.00			

* ;significant difference among rainy, winter and summer

^{ns} ;significant difference among rainy, winter and summer

To compare the estrogenic activity of *P. mirifica* of the two cultivars(PM-III and PM-IV), the length of the appearance of cornified cells after *P. mirifica* treatment and post-treatment were compared. Samples collected in rainy season were statistical difference from the others. The dose of 100 and 1,000 mg/kg BW/day of PM-III and PM-IV collected in summer tended to show the lowest estrogenic activity (the shortest duration of appearances of cornified cells), (Figure 17,18).

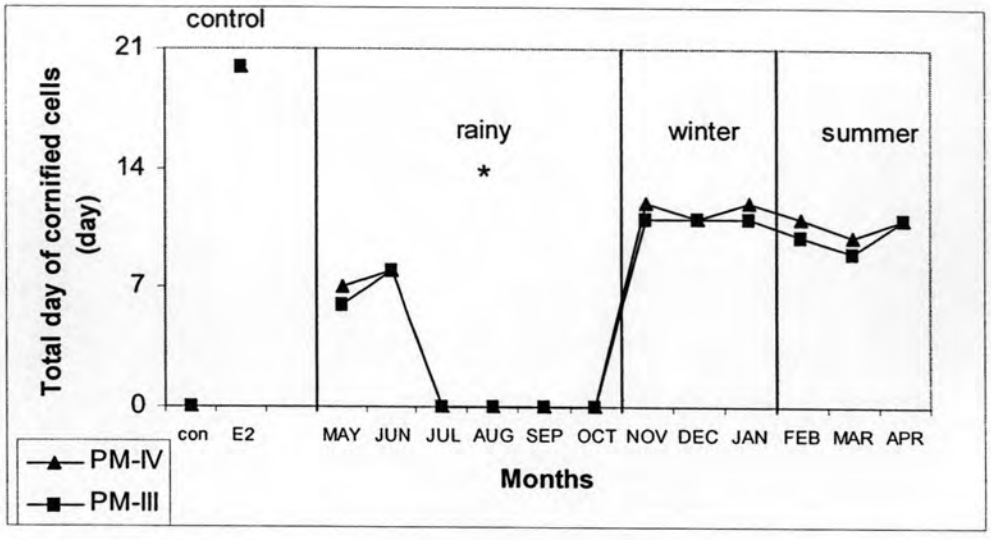


Figure 4.17 The total day of appearance of cornified cells during treatment and post-treatment periods in the dose of 100 mg/kg BW/day of PM-III and PM-IV (* ;significant difference among rainy, winter and summer)

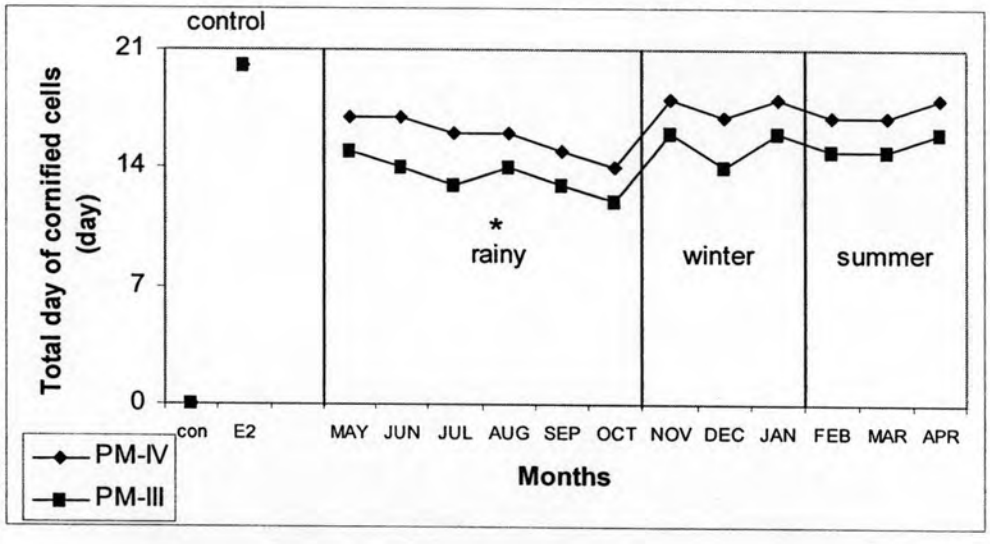


Figure 4.18 The total day of appearance of cornified cells during treatment and post-treatment periods in the dose of 1,000 mg/kg BW/day of PM-III and PM-IV (* ;significant difference among rainy, winter and summer)

To compare the estrogenic activity of *P. mirifica* of the two doses, the length of the appearance of cornified cells after *P. mirifica* treatment and post-treatment were compared. At the dosage of 100 mg/kg BW/day, it was significant longer than the negative control and significantly shorter than the positive control. At the dose of 1,000 mg/kg BW/day, the rats exhibited the highest estrogenic activity but was still significantly lower than the positive control ($P<0.05$) (Figure 4.19).

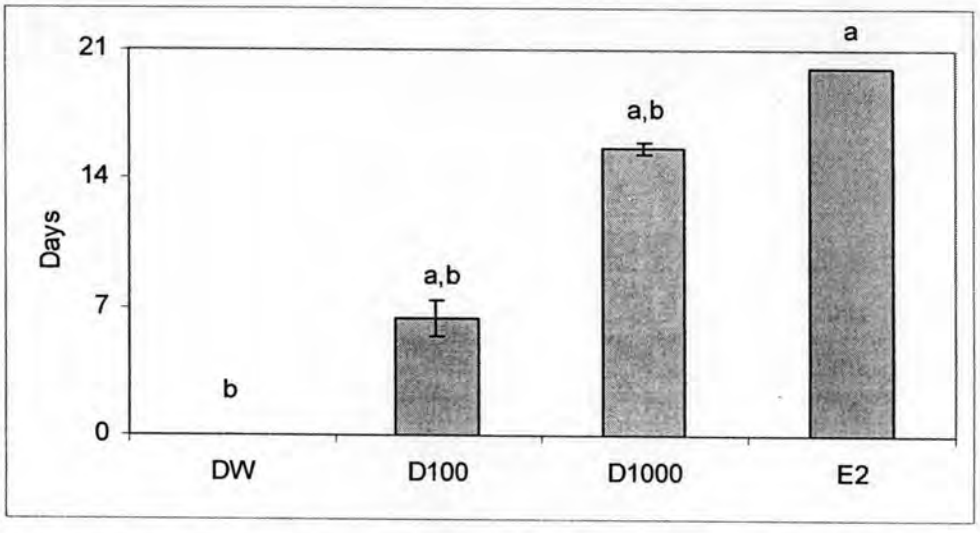


Figure 4.19 The mean ± S.E.M. of the length of the appearance of cornified cells during the 21-day period of *P. mirifica* treatment and post-treatment.

a; significant differences compared with the negative control (distilled water).

b; significant differences compared with the positive control (17β - estradiol).

4.3 Influence of *P. mirifica* (PM-III and PM-IV) on the percentage of cornified cells in ovariectomized rats

From the result of vaginal cornification in rats treated with 100 mg/kg BW/day of *P. mirifica,* it was found that the dosage of 100 mg/kg BW/day of PM-III, tended to show lower estrogenic activity (slower appearance of cornified cells) than PM-IV. It was found that the earliest response was on D_6 after treated with PM-III collected in January, April, November, and December. And the vaginal cornification in rats after treated with 100 mg/kg BW/day of PM-IV exhibited the earliest response on D_5 after treated with PM-IV collected in January and November. The latest response of vaginal cornification was found on D_4 in rats treated with 1,000 mg/kg BW/day of PM-III collected in July, September and October. The latest response of vaginal

cornification was found on D_3 in rats treated with 1,000 mg/kg BW/day of PM-IV collected from July and October. These results agreed with the day of appearance of leucocytes cells during post-treatment period and the duration of appearance of cornified cells during both treatment and post-treatment periods.

To rank the highest and lowest estrogenic activity in 2 cultivars of *P. mirifica*, the vaginal smear cells at the first day of appearance of cornified cells were counted and calculated for the percentage of cornified cells. One-hundred vaginal smear cells were randomly counted for all of Co, O and L cell types. The percentage of cornified cells was calculated. Figure 4.20, 4.21, 4.22 and 4.23 presented mean ± S.E.M. of the percentage of cornified cells from 5 rats treated with *P. mirifica* (PM-III and PM-IV) compared with the positive and negative control groups.

To find out the highest estrogenic activity of PM-III, the percentage of cornified cells counted on D_6, the soonest first day of appearance of cornified cells after 100 mg/kg BW/day of PM-III treatment was compared between January, November, December and April. It was found that the percentage of cornified cells in rats after feeding with PM-III collected in January (89.00 ± 1.76) was higher than November (86.80 ± 0.86), December (84.40 ± 0.87) and April (82.20 ± 0.86). The estrogenic activity of PM-III collected in January was the highest of the 12 month samples. The percentage of cornified cells counted on D_5, the soonest first day of appearance of cornified cells after 100 mg/kg BW/day of PM-IV treatment was compared between January and November. It was found that the percentage of cornified cells after feeding with PM-IV collected in January (88.60 ± 1.47) was higher than November (83.20 ± 1.46). Consider from the first day of appearance of cornified cells, the dosage of 100 mg/kg BW/day of PM-IV exhibited stronger estrogenic activity (earlier appearance of cornified cells) than PM-III.

However, the percentage of cornified cells counted on D_6 (PM-III) and D_5 (PM-IV) after feeding with 100 mg/kg BW/day of 2 cultivars of *P. mirifica* collected in January was still lower than that of positive control which exhibited one hundred percent of cornified cells. In addition, the percentage of cornified cells count on D_4 after 1,000 mg/kg BW/day of PM-III treatment was compared between July, September and October, for the lowest estrogenic activity. It was found that the percentage of cornified cells in rats after feeding with PM-III collected from October (83.40 ± 1.54) was lower than July (86.20 ± 1.36) and September (91.40 ± 2.42). The

estrogenic activity of PM-III collected in October was the lowest of 12 months studied period. The percentage of cornified cells count on D_3 after 1,000 mg/kg BW/day of PM-IV treatment was compared between July and October. It was found that the percentage of cornified cells in rats feeding with PM-IV collected from October (83.00 ± 0.70) was lower than July (91.00 ± 1.41). That is the estrogenic activity of PM-IV collected in October was the lowest among the 12 month samples.

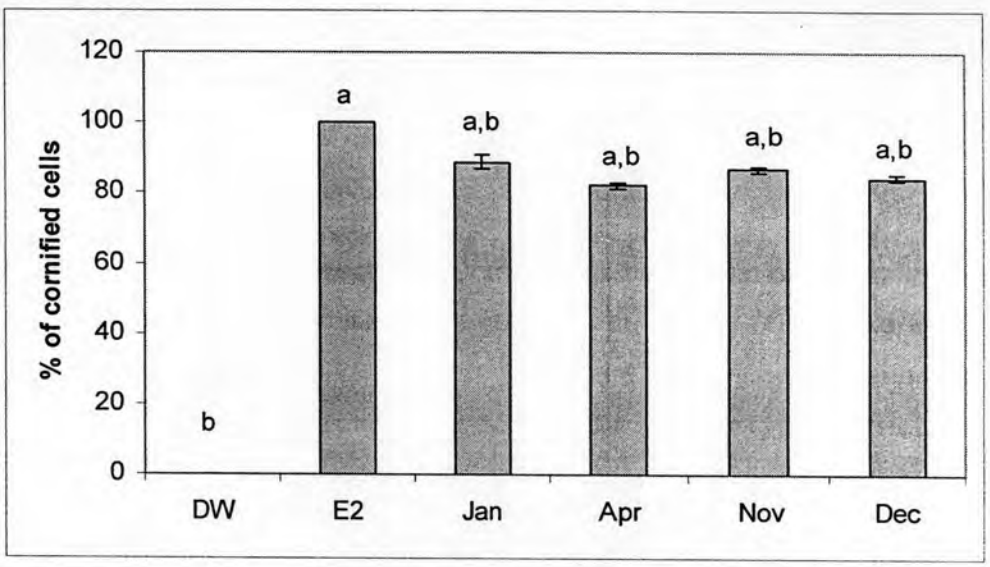


Figure 4.20 The percentage of cornified cell in rats counted on D_6 after feeding of PM-III in the dose of 100 mg/kg BW/day, distilled water and 17β-estradiol.
a; significant differences compared with the negative control (distilled water).
b; significant differences compared with the positive control (17β-estradiol).

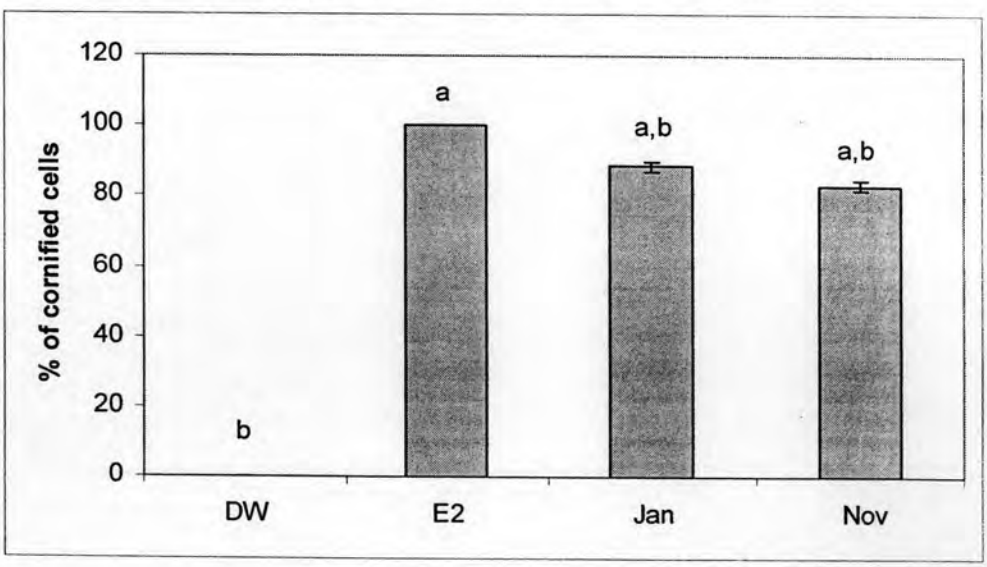


Figure 4.21 The percentage of cornified cell in rats counted on D_5 after feeding of PM-IV in the dose of 100 mg/kg BW/day, distilled water and 17β-estradiol.

a; significant differences compared with the negative control (distilled water).
b; significant differences compared with the positive control (17β-estradiol).

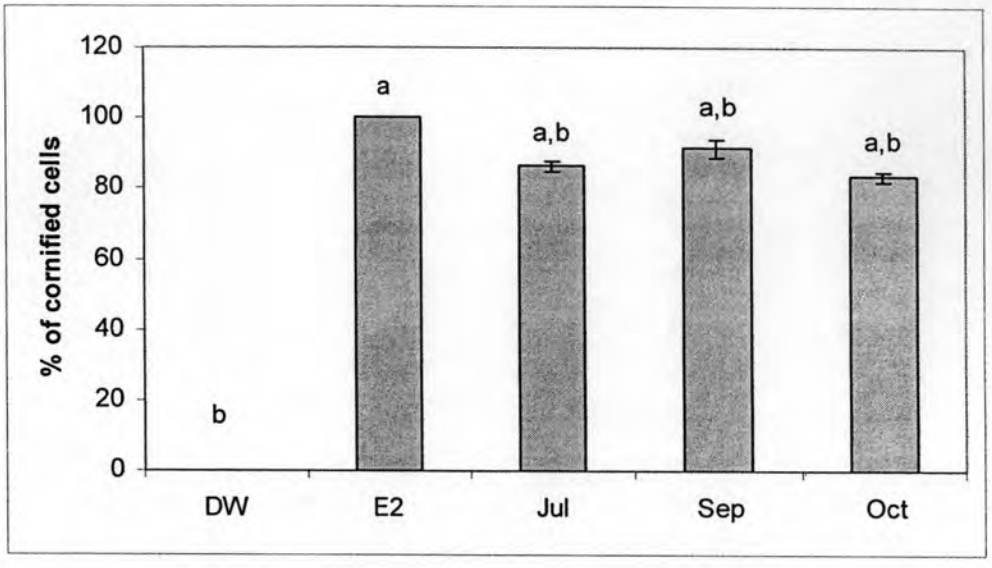


Figure 4.22 The percentage of cornified cell in rats counted on D_4 after feeding of PM-III in the dose of 1,000 mg/kg BW/day, distilled water and 17β-estradiol.
a; significant differences compared with the negative control (distilled water).
b; significant differences compared with the positive control (17β-estradiol).

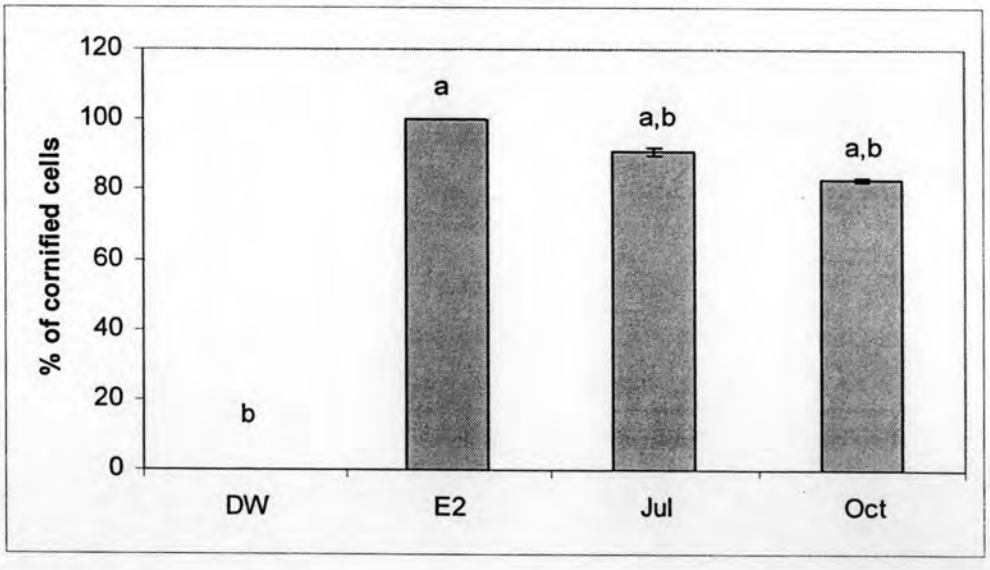


Figure 4.23 The percentage of cornified cell in rats counted on D_3 after feeding of PM-IV in the dose of 1,000 mg/kg BW/day, distilled water and 17β-estradiol.
a; significant differences compared with the negative control (distilled water).
b; significant differences compared with the positive control (17β-estradiol).

4.4 Effect of *P. mirifica* on body weight of ovariectomized rats

The rat body weights in all groups were not significant difference during the pretreatment period (day 1 and 7) compared to the negative control group. During the *P. mirifica* treatment, the rat body weight was decreased in a dose-dependent manner. The body weight changes in each group are as follows;

4.4.1 Control groups

Negative control: The body weight of rats in the negative control group was significantly increased from day 1 presented in Figure 4.24.

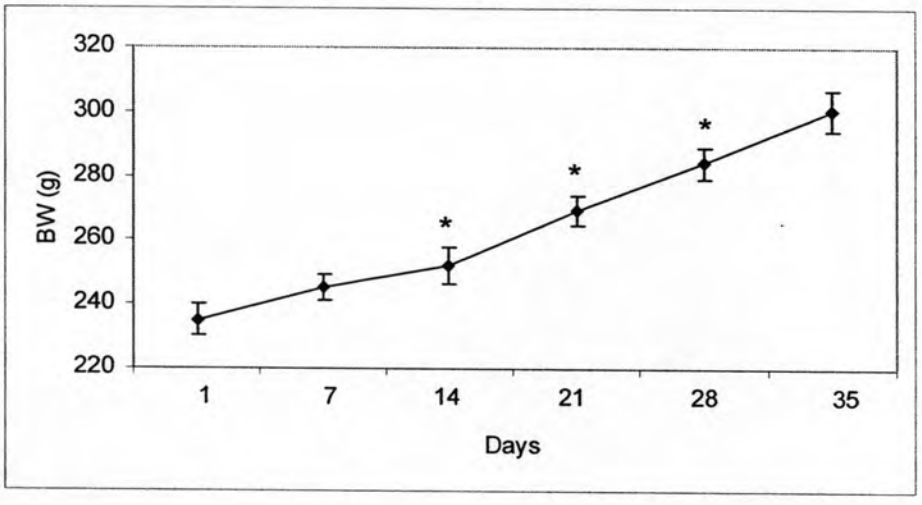


Figure 4.24 The body weight of rat treated with distilled water (* =significant difference from day 1).

Positive control (17β-estradiol): The body weight of rats in the positive control group compared to the negative control group was significantly increased as presented in Figure 4.25.

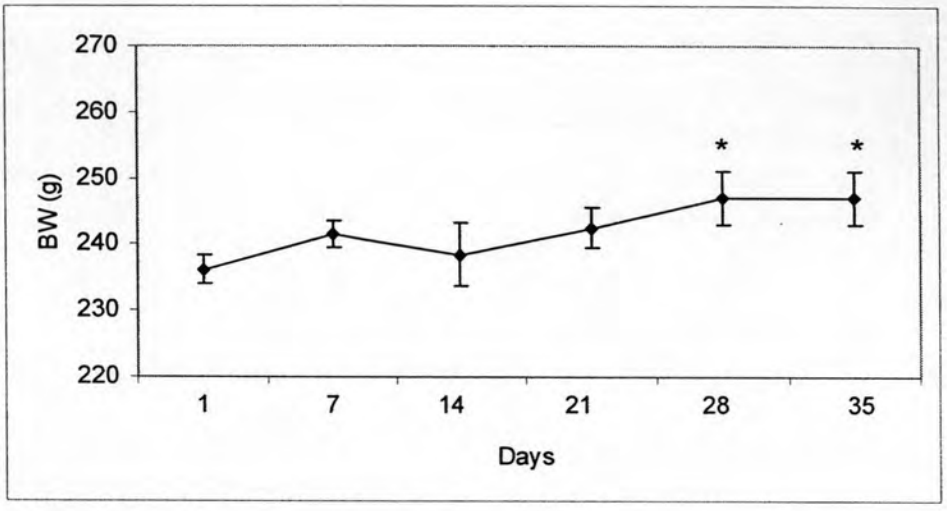


Figure 4.25 The body weight of rat treated with the 17β -estradiol (* =significant difference from day 1).

4.4.2 *P. mirifica* treatement groups

Dose 100 mg/kg BW/day

The body weight were significant decreased from the control group in the day 7, 14, 21, 28, 35 ($P<0.05$), (Figure 4.26).

Dose 1,000 mg/kg BW/day

The body weight were significant decreased from the control group in the day 7, 14, 35 ($P<0.05$), (Figure 4.27).

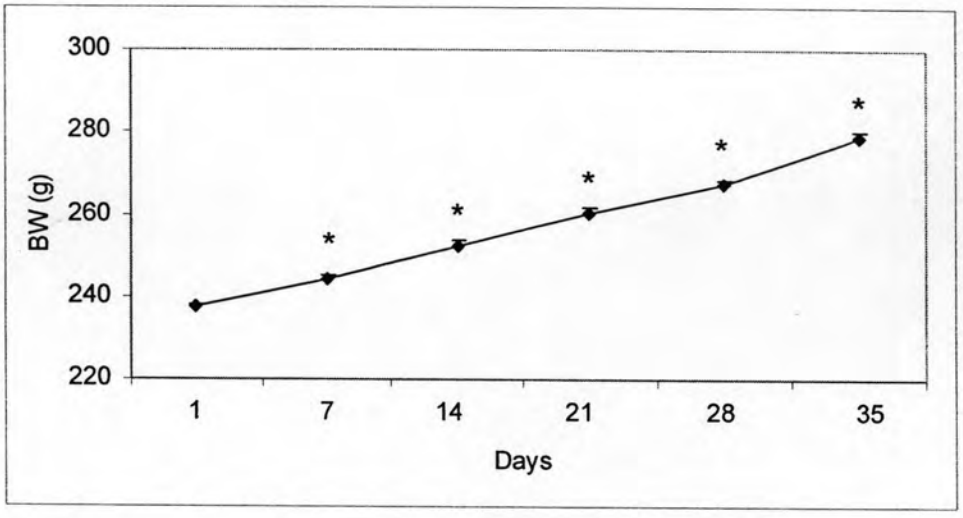


Figure 4.26 The body weight of rat treated with 100 mg/kg BW/day of *P. mirifica* on day 1, 7, 14, 21, 28, 35, 42 (* =significant difference from negative control).

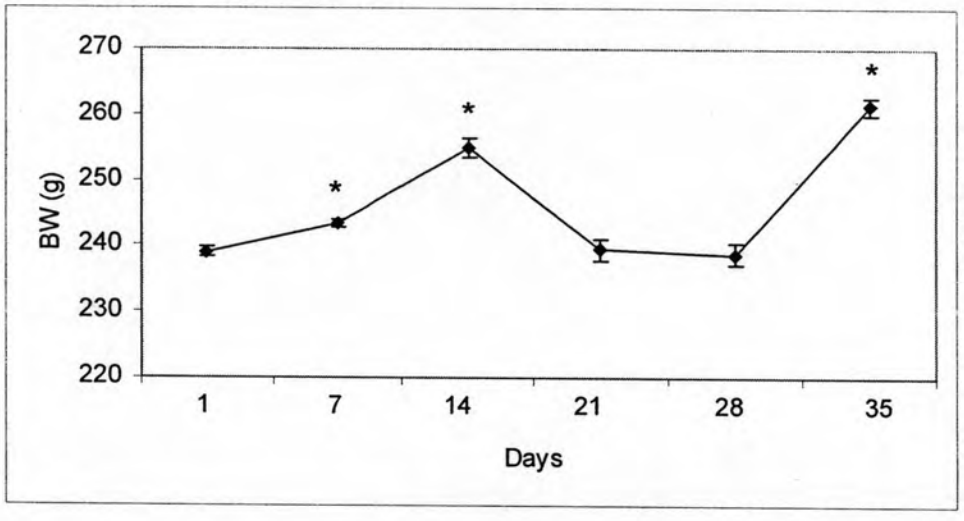


Figure 4.27 The body weight of rat treated with 1,000 mg/kg BW/day of *P. mirifica* on day 1, 7, 14, 21, 28, 35, 42 (* =significant difference from negative control).

4.5 Effect of *P. mirifica* on uterine weights of ovariectomized rats

The result of uterine weight in rats treated with *P. mirifica* (PM-III and PM-IV) 17β-estradiol and distilled water are presented in Table 4.4 and 4.5. The increment of uterine weight at the end of the treatment period (day 28th) and post- treatment period (day 35 th) was agreed with changes of vaginal epithelium cell. The uterine weights in rat treated with 100 mg/kg BW/day of PM-IV collected in summer season was significantly higher than that of the winter and rainy. The uterine weight in rats treated with 100 mg/kg BW/day of PM-IV were as follows; summer> winter ≥ rainy. However, there were no significant differences of uterine weights in rats treated with 100 mg/kg BW/day of PM-III collected in those 3 seasons (Table 4.4). The uterine weight in rats treated with 1,000 mg/kg BW/day of PM-IV in post-treatment period collected in rainy season was significantly lower than that of the summer and winter. The uterine weight in rats treated with 100 mg/kg BW/day of PM-IV were as follows; winter ≥ summer > rainy.

The increment of uterine weights also depended on the dose of treatment, for instance, the uterine weight gain of rats treated with the dose of 1,000 mg/kg BW/day of PM-III was greater than the treatment with the dose of 100 mg/kg BW/day, respectively. The increment of uterine weights also depended on the cultivars of *P. mirifica*. The uterine weight gain of rats treated with 1,000 mg/kg BW/day of *P. mirifica* cultivar PM-IV were also greater than the dose 1,000 mg/kg BW/day PM-III.

The uterine weight gain of rats treated with the dose of 1,000 mg/kg BW/day of sample that collected in rainy season were statistical difference from the others in the dose of 1,000 mg/kg BW/day of PM-III and PM-IV (Table 4.5).

When the data of uterine weights of rats treated with PM-III and PM-IV collected in 12 months were pooled and compared between doses, the uterine weight was increased in a dose dependent manner as presented in Figure 4.28. The uterine weight of rats treated with 100 and 1,000 mg/kg BW/day of *P. mirifica* showed significantly differ from that of the negative and positive control group.

Table 4.4 The uterine weights of rats treated with 100 mg/kg BW/day of *P. mirifica* cultivar PM-III and PM-IV at the end of post-treatment period (day 35th).

season	month	PM-III 100 mg/kg BW/day	PM-IV 100 mg/kg BW/day
Rainy	May	174.00 ± 14.82	191.80 ± 6.22
	June	172.30 ± 4.22	220.50 ± 6.55
	July	154.80 ± 14.39	171.60 ± 3.80
	August	178.20 ± 7.07	187.60 ± 3.40
	September	180.80 ± 3.38	194.60 ± 21.95
	October	160.80 ± 2.96	183.40 ± 4.77
mean ± S.E.M. (n=6)		170.15 ± 4.17 $^{a, b}$	191.58 ± 6.65 $^{a, b}$
Winter	November	211.20 ± 24.51	212.80 ± 4.62
	December	160.60 ± 7.22	197.50 ± 20.43
	January	204.80 ± 6.70	199.80 ± 10.02
mean ± S.E.M. (n=3)		192.20 ± 15.91 $^{a, b}$	203.37 ± 4.76 $^{a, b}$
Summer	February	229.20 ± 9.65	244.00 ± 11.69
	March	194.80 ± 10.45	221.40 ± 20.49
	April	197.80 ± 5.41	226.60 ± 5.21
mean ± S.E.M. (n=3)		207.27 ± 11.00 $^{a, b}$	230.67 ± 6.83 $^{*,a, b}$
Control groups	DW	132.80 ± 1.39 ^{b}	
	E2	537.3 ± 14.16 ^{a}	

* ; significant difference among rainy, winter and summer

a; significant difference compared with the negative control (distilled water).

b; significant difference compared with the positive control (17β - estradiol).

Table 4.5 The uterine weights of rats treated with 1,000 mg/kg BW/day of *P. mirifica* cultivar PM-III and PM-IV at the end of treatment period (day 28 th) and post-treatment period (day 35th).

season	month	Treatment period		Post-treatment period	
		PM-III 1,000 mg/kg BW/day	PM-IV 1,000 mg/kg BW/day	PM-III 1,000 mg/kg BW/day	PM-IV 1,000 mg/kg BW/day
Rainy	May	445.80 ± 18.57	489.80 ± 26.40	240.00 ± 3.70	253.80 ± 9.44
	June	503.50 ± 18.16	538.20 ± 16.45	231.00 ± 10.02	278.60 ± 11.14
	July	452.20 ± 23.57	432.60 ± 8.73	219.80 ± 2.60	236.80 ± 5.78
	August	478.40 ± 32.20	472.20 ± 30.93	227.20 ± 4.57	225.70 ± 6.96
	September	467.40 ± 24.17	483.30 ± 18.03	246.40 ± 8.03	293.40 ± 23.72
	October	456.00 ± 21.46	456.20 ± 15.29	264.60 ± 12.42	246.20 ± 2.46
mean ± S.E.M. (n=6)		467.22 ± 8.66 ^{ns}	478.72 ± 14.56*	238.17 ± 6.53 ^{ns}	255.75 ± 10.48*
Winter	November	510.00 ± 16.29	579.60 ± 68.43	290.00 ± 11.42	334.60 ± 34.72
	December	364.40 ± 30.89	552.50 ± 20.70	231.20 ± 9.15	316.40 ± 19.05
	January	536.00 ± 36.35	589.60 ± 45.22	275.40 ± 8.62	367.20 ± 18.83
mean ± S.E.M. (n=3)		470.13 ± 53.40 ^{ns}	573.90 ± 11.08	265.53 ± 17.68 ^{ns}	339.40 ± 14.86
Summer	February	546.00 ± 21.20	552.20 ± 30.83	317.80 ± 11.46	415.60 ± 47.89
	March	446.00 ± 14.39	589.60 ± 64.02	251.00 ± 13.01	285.20 ± 9.71
	April	470.60 ± 10.76	536.80 ± 30.64	250.40 ± 2.87	287.40 ± 4.14
mean ± S.E.M. (n=3)		487.53 ± 30.08 ^{ns}	559.53 ± 15.68	273.06 ± 22.37 ^{ns}	329.40 ± 43.10
Control groups	DW	136.60 ± 1.21		132.80 ± 1.39	
	E2	1152.00 ±1 9.14		537.3 ± 14.16	

* ; significant difference among rainy, winter and summer

^{ns} ; significant difference among rainy, winter and summer

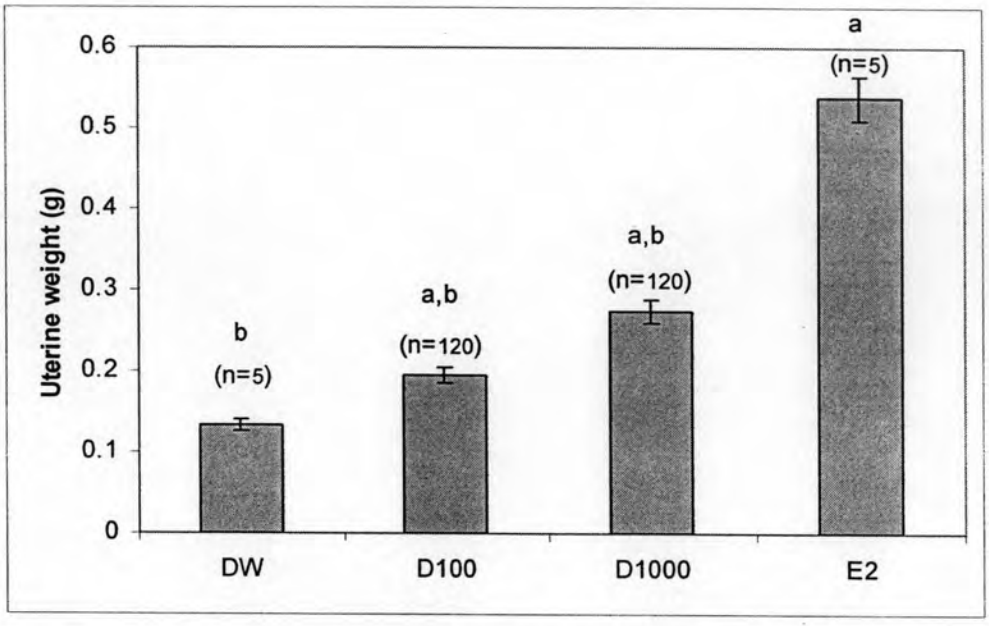


Figure 4.28 The rat uterine weight in the control and treatment group.

a; significant difference compared with the negative control (distilled water).

b; significant difference compared with the positive control (17β - estradiol).

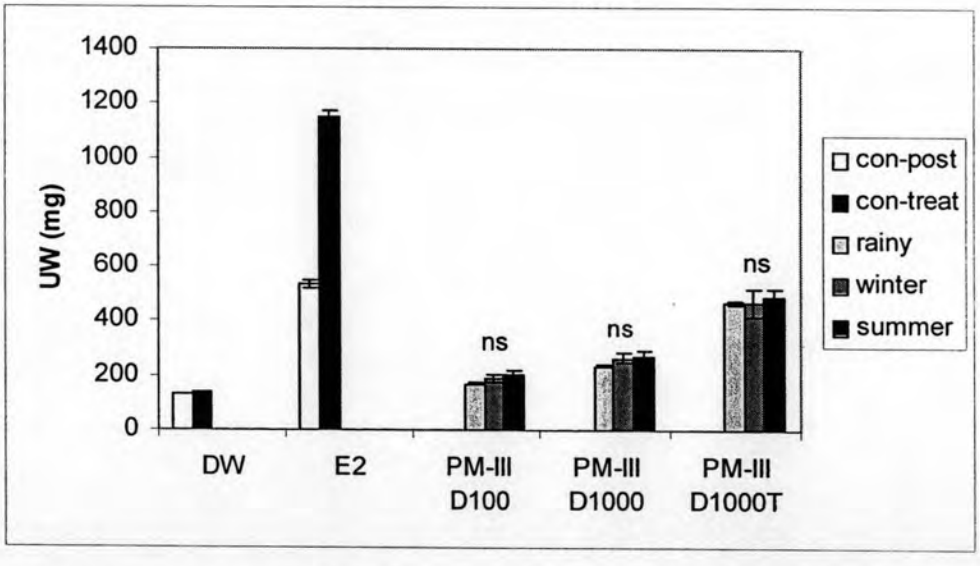


Figure 4.29 The rat uterine weight in the control and the PM-III treatment group.

^{ns} ; significant difference among rainy, winter and summer

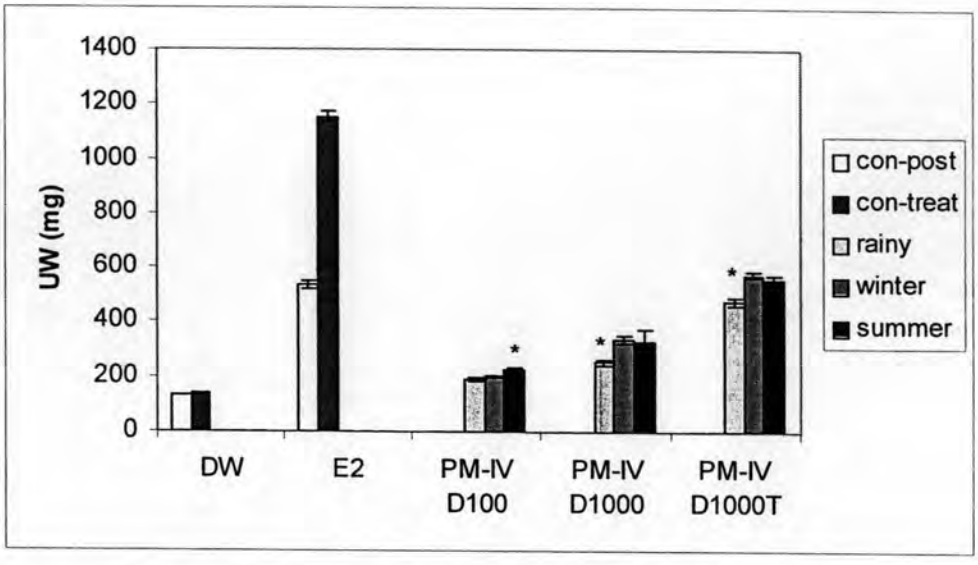


Figure 4.30 The rat uterine weight in the control and the PM-IV treatment group.
* ; significant difference among rainy, winter and summer

4.6 Effect of *P. mirifica* on uterine gland number of ovariectomized rats

Uterus was submitted to histology preparation and analysis. The key used parameter is uterine gland number. The result of uterine gland number in rats treated with *P. mirifica* (PM-III and PM-IV), 17β-estradiol and distilled water are presented in Table 4.6 and Figure 29,30. The increment of uterine gland number at the end of the treatment period (day 28th) was agreed with changes of vaginal epithelium cell. The uterine gland number in the uterine tissue of rat treated with 1,000 mg/kg BW/day of PM-III and PM-IV collected in rainy was significantly lower than that the winter and summer season (Table 4.6).

The uterine gland number in the uterine tissue of rat treated with 1,000 mg/kg BW/day of PM-III was as follows; winter ≥ summer > rainy, PM-IV was as follows; winter ≥ summer > rainy, too. However, there were no significant differences of uterine gland number in uterine tissue of rats treated with 1,000 mg/kg BW/day of PM-III and PM-IV collected in winter and summer seasons (Figure 4.29, 4.30).

The increment of uterine glands also depended on the cultivars of *P. mirifica*. The uterine glands in uterine tissue of rats treated with 1,000 mg/kg BW/day of *P. mirifica* cultivar PM-IV was more than PM-III (Table 4.6).

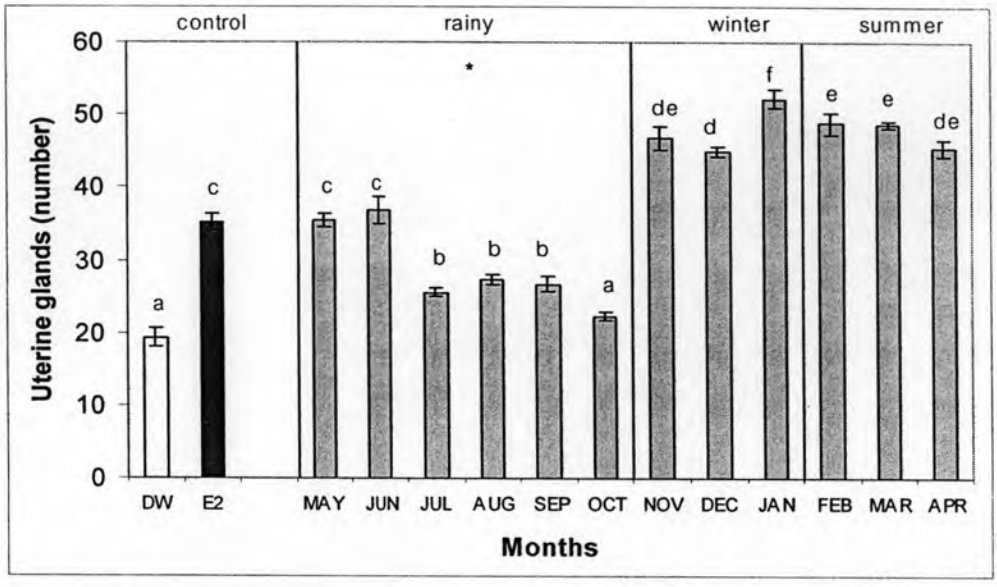


Figure 4.31 The number of uterine gland at the dose of 1,000 mg/kg BW/day of PM-III (* :significant difference among rainy, winter and summer)
Means not sharing a common superscript letter in the same column of one province are significantly different ($P < 0.05$) as determined by Duncan's multiple range test.

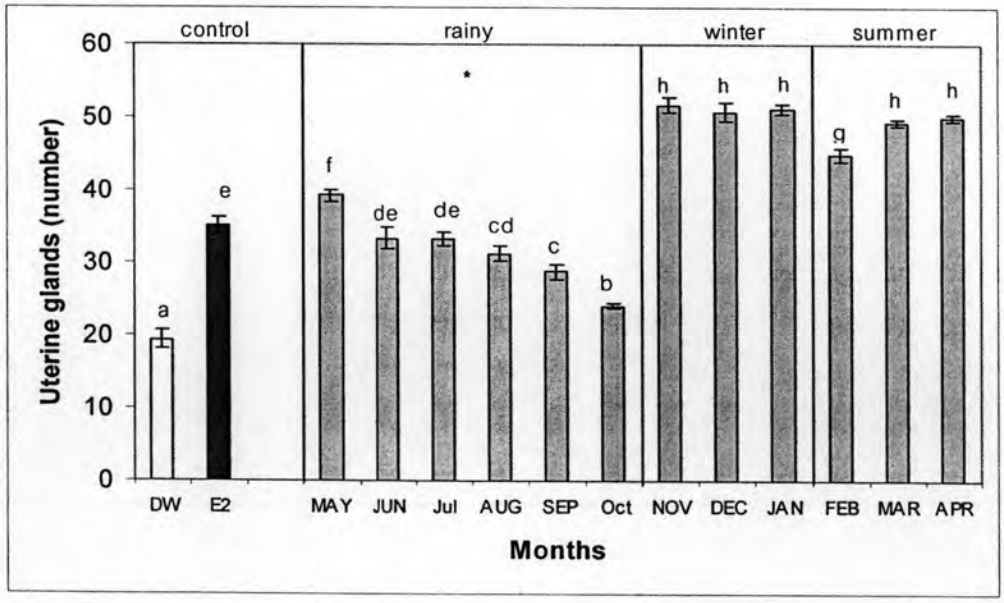


Figure 4.32 The number of uterine gland at the dose of 1,000 mg/kg BW/day of PM-IV (* :significant difference among rainy, winter and summer)

Means not sharing a common superscript letter in the same column of one province are significantly different ($P < 0.05$) as determined by Duncan's multiple range test.

Table 4.6 The uterine gland number of rats treated with 1,000 mg/kg BW/day of *P. mirifica* cultivar PM-III and PM-IV at the end of treatment period (day 28th)

season	month	PM-III 1000 mg/kg BW/day	PM-IV 1000 mg/kg BW/day
Rainy	May	35.44 ± 1.00	39.22 ± 0.82
	June	36.89 ± 1.81	33.28 ± 0.89
	July	25.56 ± 0.61	24.06 ± 0.39
	August	27.33 ± 0.65	31.22 ± 1.07
	September	26.67 ± 1.05	28.72 ± 1.16
	October	22.33 ± 0.62	33.33 ± 1.58
mean ± S.E.M. (n=6)		29.04 ± 2.37*, a, b	31.64 ± 2.08*, a, b
Winter	November	46.78 ± 1.54	51.22 ± 0.67
	December	44.83 ± 0.70	50.72 ± 1.28
	January	52.22 ± 1.31	51.72 ± 0.98
mean ± S.E.M. (n=3)		47.94 ± 2.21a, b	51.22 ± 0.29 a, b
Summer	February	48.83 ± 1.52	44.89 ± 1.04
	March	48.61 ± 0.51	49.22 ± 0.45
	April	45.44 ± 1.14	50.06 ± 0.49
mean ± S.E.M. (n=3)		47.63 ± 1.09 a, b	48.06 ± 1.60 a, b
Control groups	DW	19.33 ± 1.25 b	
	E2	35.17 ± 1.21 a	

* ; significant difference among rainy, winter and summer

a; significant difference compared with the negative control (distilled water).

b; significant difference compared with the positive control (17β - estradiol).

When the data of uterine glands in uterine tissue of rats treated with 1,000 mg/kg BW/day of PM-III and PM-IV collected in 12 months were pooled and compared between cultivars, the uterine glands number also depended on the cultivars as presented in Figure 4.31. The uterine glands in uterine tissue of rats treated with 1,000 mg/kg BW/day of *P. mirifica* cultivar PM-IV was more than PM-III.

The uterine glands number in uterine tissue of rats treated with PM-IV *P. mirifica* showed significantly differ from that of the negative and positive control group , however PM-III showed no significantly differ from that of the positive control group but showed significantly differ from that of the negative control (Figure 4.31).

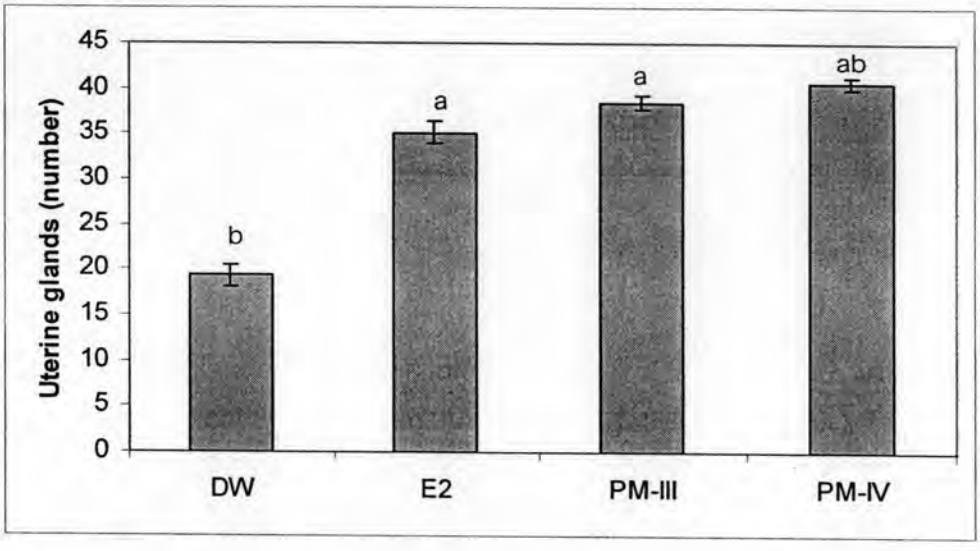


Figure 4.33 The number of uterine gland at the dose of 1,000 mg/kg BW/day of PM-III and PM-IV.

a; significant difference compared with the negative control (distilled water).

b; significant difference compared with the positive control (17β - estradiol).

4.7 Effect of *P. mirifica* on the increment of uterine tissue of ovariectomized rats

Qualitative analysis is submitted to analyze the cross section area of uterine tissue is expressed into 3 parts of the uterus, including myometrium, endometrium and lumen (Table 4.7, 4.8) In PM-III there is no difference in cross section area of myometrium. It means that estrogenic effect is not much influence to myometrium. Endometrium treated with plant samples collected in winter is thicker than in rainy season and summer. Estrogenic effect to uterus is thus dominated at endometrium. Lumen treated with plant samples collected in rainy season is larger than the other 2 seasons. There is thus a correlation change of endometrium and lumen (Table 4.7). In PM-IV, myometrium shows estrogenic response. It is thicker in winter than summer and rainy seasons (Table 4.8). It means that PM-IV expresses stronger estrogenic effect to myometrium than PM-III. Endometrium is thicker in winter than

summer and rainy season. This is the additional evidence that PM-IV has stronger estrogenic activity than PM-III. Lumen is larger in rainy season than winter and summer. It confirms that the enlargement of lumen should result from the reduction in thickness of endometrium in rainy season.

Table 4.7 The cross section area of uterine tissue of rats treated with 1,000 mg/kg BW/day of *P. mirifica* cultivar PM-III at the end of treatment period (day 28)

season	month	PM-III		
		Myometrium (mm^2)	Endometrium (mm^2)	Lumen (mm^2)
rainy	May	0.13425 ± 0.00298	0.09475 ± 0.00351	0.06650 ± 0.00127
	June	0.13375 ± 0.00698	0.12050 ± 0.0005	0.01100 ± 0.00128
	July	0.09125 ± 0.00195	0.08800 ± 0.00076	0.06775 ± 0.00244
	August	0.13675 ± 0.00949	0.09825 ± 0.00565	0.03400 ± 0.00131
	September	0.12800 ± 0.00693	0.10325 ± 0.0028	0.01525 ± 0.00409
	October	0.09375 ± 0.00309	0.06600 ± 0.00335	0.06750 ± 0.0068
mean ± S.E.M. (n=6)		0.1195± 0.00347 $^{ns, a}$	0.09525± 0.00294 $^{ns, a}$	0.04375± 0.00440 $^{ns, a, b}$
Winter	November	0.15450 ± 0.05240	0.12875 ± 0.00189	0.02225 ± 0.00442
	December	0.12650 ± 0.00800	0.09925 ± 0.00162	0.00825 ± 0.0005
	January	0.15800 ± 0.00463	0.14525 ± 0.00175	0.00575 ± 0.00019
mean ± S.E.M. (n=3)		0.14625± 0.00399 $^{ns, a}$	0.1245± 0.00538 $^{ns, a, b}$	0.01200± 0.00205 $^{ns, b}$
summer	February	0.13450 ± 0.00342	0.09225 ± 0.00316	0.00900 ± 0.00076
	March	0.15000 ± 0.00582	0.08850 ± 0.00211	0.02050 ± 0.00266
	April	0.15325 ± 0.00325	0.11450 ± 0.00723	0.00625 ± 0.00078
mean ± S.E.M. (n=3)		0.14600± 0.00231 $^{ns, a}$	0.09850± 0.00325 $^{ns, a}$	0.01200± 0.00175 $^{ns, b}$
control groups	DW	0.04300± 0.00137 ^{b}	0.01850± 0.0006 ^{b}	0.00125± 0.00005 ^{b}
	E2	0.12025± 0.00137 ^{a}	0.08075± 0.00467 ^{a}	0.1875± 0.00742 ^{a}

* ; significant difference among rainy, winter and summer

a; significant difference compared with the negative control (distilled water).

b; significant difference compared with the positive control (17β - estradiol).

Table 4.8 The cross section area of uterine tissue of rats treated with 1,000 mg/kg BW/day of *P. mirifica* cultivar PM-III at the end of treatment period (day 28)

season	month	PM-IV		
		Myometrium (mm^2)	Endometrium (mm^2)	Lumen (mm^2)
rainy	May	0.12000 ± 0.00056	0.09800 ± 0.00175	0.06300 ± 0.00394
	June	0.12750 ± 0.00273	0.11900 ± 0.00092	0.01475 ± 0.0022
	July	0.10225 ± 0.00507	0.09400 ± 0.00129	0.07575 ± 0.00433
	August	0.12475 ± 0.00212	0.10050 ± 0.00373	0.07225 ± 0.00713
	September	0.11525 ± 0.0048	0.11200 ± 0.00772	0.03725 ± 0.00521
	October	0.09400 ± 0.0033	0.09400 ± 0.00129	0.07850 ± 0.00604
mean ± S.E.M. (n=6)		0.13650± 0.00216*, a	0.10300±0.00168*, a ,b	0.05700± 0.0041*, a, b
winter	November	0.15600 ± 0.00292	0.15150 ± 0.00385	0.01900 ± 0.00193
	December	0.13250 ± 0.00658	0.13800 ± 0.00163	0.00950 ± 0.00033
	January	0.16325 ± 0.00408	0.15225 ± 0.00246	0.01275 ± 0.00087
mean ± S.E.M. (n=3)		0.15050± 0.00371 a	0.14725 ± 0.00185*, a ,b	0.01375 ± 0.00112 b
summer	February	0.14650 ± 0.00566	0.11750 ± 0.00199	0.01075 ± 0.00131
	March	0.15375 ± 0.00261	0.12100 ± 0.00092	0.02250 ± 0.00559
	April	0.13925 ± 0.0058	0.00310 ± 0.00696	0.00775 ± 0.00034
mean ± S.E.M. (n=3)		0.14650 ± 0.00167 a	0.12500± 0.00234*, a ,b	0.01375 ± 0.00180 b
control groups	DW	0.04300± 0.00137 b	0.01850± 0.0006 b	0.00125± 0.00005 b
	E2	0.12025± 0.00137 a	0.08075± 0.00467 a	0.1875± 0.00742 a

* ; significant difference among rainy, winter and summer

a; significant difference compared with the negative control (distilled water).

b; significant difference compared with the positive control (17β - estradiol).

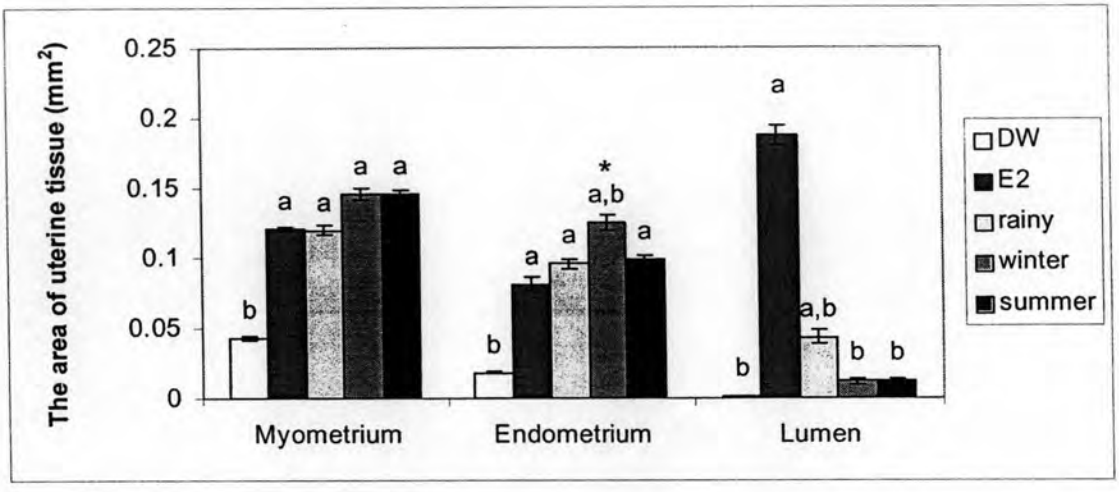


Figure 4.34 The area of uterine tissue at the dose of 1,000 mg/kg BW/day of PM-III

* ; significant difference among rainy, winter and summer

a; significant difference compared with the negative control (distilled water).

b; significant difference compared with the positive control (17β - estradiol).

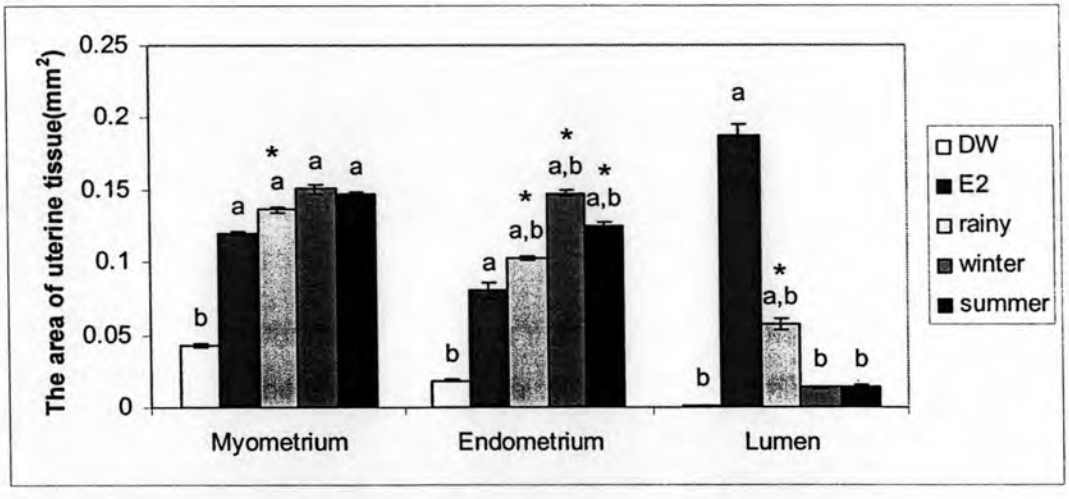


Figure 4.35 The area of uterine tissue at the dose of 1,000 mg/kg BW/day of PM-IV

* ; significant difference among rainy, winter and summer

a; significant difference compared with the negative control (distilled water).

b; significant difference compared with the positive control (17β - estradiol).

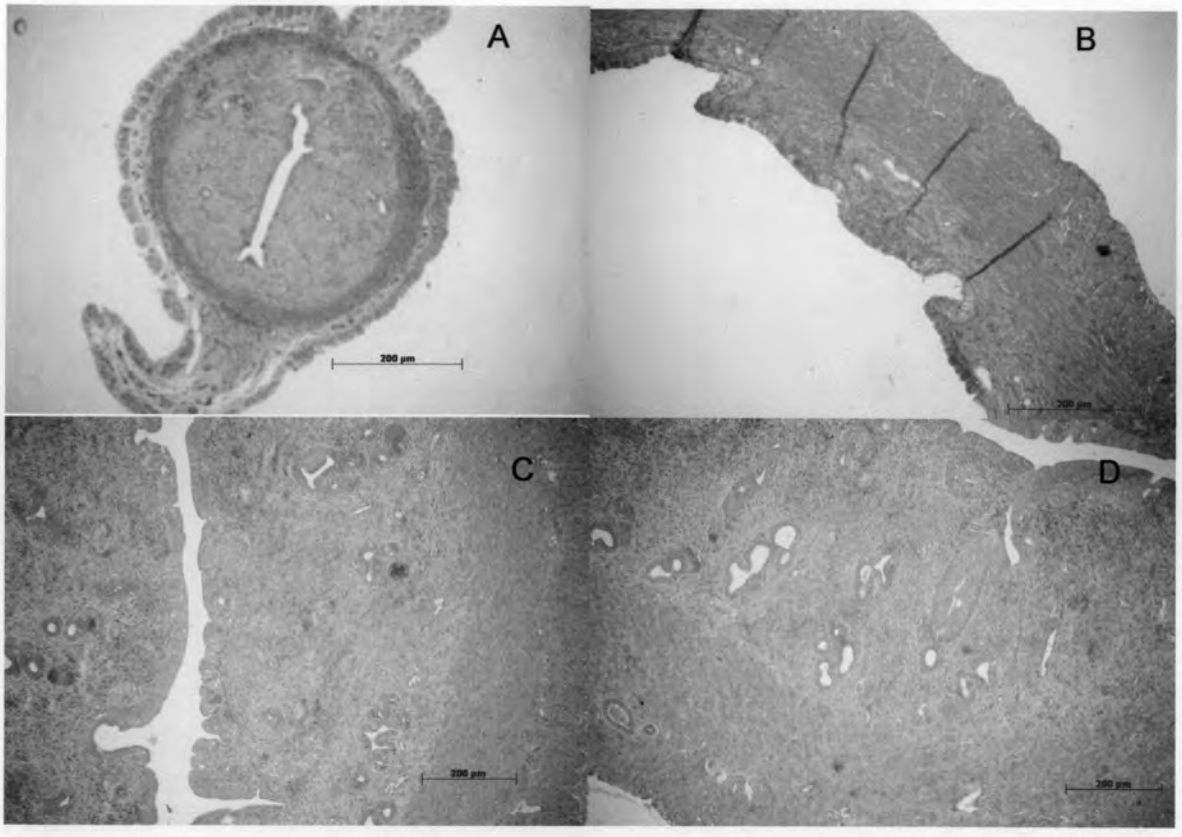


Figure 4.36 Histology of uterine tissue of OVX rats at the dose of 1,000 mg/kg BW/day of the treatment with distilled water: A , E2: B, PM-III: C, PM-IV: D. (The scale bar represented 10 μm).

4.8 The correlation analysis between isoflavone content of *P. mirifica* (PM-III and PM-IV) and the vaginal cornification assay, uterotrofic assay, uterine gland number assay and the cross section area of uterine tissue assay.

The result of estrogenic activity of *P. mirifica* that is determined by the vaginal cornification assay, uterotrofic assay, uterine gland number assay and the cross section area of uterine tissue assay and the isoflavone content of PM-III and PM-IV. The 12 samples of PM-III and PM-IV in vaginal cornification are correlated with the total isoflavone or individual isoflavone contents including puerarin, daidzin, genistin, daidzein and genistein ($P \leq 0.05$, $P \leq 0.01$) (Table 4.9). It was found that no correlation between estrogenic activities determine by virginal cornification assay and the Isoflavonoid contents of both PM-III and PM-IV.

Table 4.9 The correlation between estrogenic activity of **PM-III sample** determine by : (vaginal cornification assay, uterotrofic assay, uterine gland number assay and the cross section area of uterine tissue assay) and the Isoflavonoid contents in mg/100 g powder of monthly collected from Ratchaburi since March 2005 to February 2006 (mean ± S.E.M.)

month	puerarin	daidzin	genistin	daidzein	genistein	total
March	31.28±2.11^{a*}	30.36±1.86^{bcd*}	9.34±0.44^{a*}	24.65±0.78^{f}	3.85±0.31^{f}	99.48±3.99^{ab*}
April	66.74±5.05^{abc*}	42.90±0.61^{bcd*}	17.18±4.85^{bc}	9.93±0.30^{cd*}	0.49±0.15^{ab*}	137.24±10.67^{bc*}
May	73.21±8.36ab^{cdef*}	43.63±9.45^{cd*}	11.33±0.68^{a*}	9.54±0.97^{cd*}	0.12±0.04^{a*}	137.83±19.13^{bcd*}
June	98.21±2.74^{f*}	42.28±3.71^{d*}	20.68±2.06^{bc}	7.04±0.85^{bc*}	0.67±0.19^{ab*}	168.89±1.00^{de}
July	129.67±14.89^{g}	23.29±5.00^{ab*}	19.88±2.58^{bc}	11.4±1.62^{d*}	1.64±0.14^{cd*}	185.88±16.74^{e}
August	56.07±5.49^{abcd*}	35.28±0.91^{bcd*}	10.75±1.13^{a*}	4.73±0.56^{ab*}	0.68±0.07^{b*}	107.51±7.32^{b*}
September	94.34±2.38^{f*}	42.09±4.20^{cd*}	13.12±1.99^{ab*}	10.18±0.69^{cd*}	0.54±0.04^{ab*}	160.27±5.61^{cde}
October	95.02±8.45^{f*}	40.33±3.96^{bcd*}	12.72±2.06^{ab*}	3.66±0.32^{a*}	2.02±0.07^{de*}	153.76±14.38^{cde}
November	85.44±19.92^{ef*}	26.00±2.81^{abc*}	12.72±1.08^{ab*}	7.51±0.80^{bc*}	1.39±0.13^{c*}	135.17±21.35^{bcd*}
December	78.09±10.66^{def*}	35.95±5.83^{bcd*}	12.72±2.00^{c}	16.86±2.16^{e*}	2.22±0.32^{e*}	155.24±16.46^{cde}
January	38.62±4.32^{ab*}	11.62±2.25^{a*}	12.72±1.41^{a*}	3.48±0.54^{a*}	0.25±0.04^{ab*}	61.75±8.44^{a*}
February	60.46±1.92^{bcde*}	78.01±11.25^{e}	12.72±2.68^{bc}	12.81±1.78^{d*}	0.72±0:09^{b*}	170.49±13.89^{de}
Correlation	No correlation					

* Less than the maximum amount at $P < 0.05$

Means not sharing a common superscript letter in the same column of one province are significantly different ($P < 0.05$) as determined by Duncan's multiple range test.

Table 4.10 The correlation between estrogenic activity of **PM-IV sample** determine by : (vaginal cornification assay, uterotrofic assay, uterine gland number assay and the cross section area of uterine tissue assay) and the Isoflavonoid contents in mg/100 g powder of monthly collected from Ratchaburi since March 2005 to February 2006 (mean ± S.E.M.)

month	puerarin	daidzin	genistin	daidzein	genistein	total
March	187.07±1.82^{e}	48.20±12.54b^{c}	21.27±2.75^{e}	14.26±0.32^{c}	0±0^{a*}	270.79±13.14^{e}
April	55.12±3.45^{a*}	27.22±3.84^{a*}	5.36±1.35^{a*}	4.38±0.67^{ab*}	0.71±0.22^{bc*}	92.80±8.25^{a*}
May	94.88±31.73^{abc*}	25.10±9.37^{a*}	4.03±1.83^{a*}	5.45±1.70^{ab*}	0.17±0.09^{a*}	129.63±44.01^{abc*}
June	104.96±8.93^{abcd*}	33.33±8.60^{ab*}	12.43±3.55^{cd*}	6.69±1.17^{ab*}	0±0^{a*}	157.40±21.55^{abcd*}
July	64.43±1.34^{a*}	30.18±6.27^{ab*}	17.83±0.21^{de}	7.59±0.21^{b*}	0.50±0.01^{ab*}	121.82±4.89^{ab*}
August	91.30±11.37^{abc*}	27.26±5.76^{a*}	7.58±1.06^{abc*}	3.69±0.44^{a*}	0.41±0.09^{abc*}	130.24±15.33^{abc*}
September	89.62±20.36^{abc*}	29.78±3.21^{ab*}	5.80±0.47^{ab*}	4.19±1.54^{a*}	0.49±0.04^{ab*}	129.89±24.83^{abc*}
October	139.37±14.44^{cd*}	35.93±3.67^{ab*}	7.97±0.79^{abc*}	4.58±0.37^{ab*}	1.79±0.06^{de*}	189.64±19.33^{bcd*}
November	92.63±14.98^{abc*}	24.05±2.83^{a*}	12.34±1.42^{cd}	6.10±0.57^{ab*}	1.57±0.03^{d*}	136.69±17.2^{abcd*}
December	173.66±26.41^{de}	30.66±3.92^{ab*}	14.73±2.84^{bcd*}	7.09±1.61^{ab*}	2.07±0.41^{e}	228.22±31.49^{d*}
January	78.00±11.03^{ab*}	15.20±2.98^{a*}	7.89±1.88^{abc*}	4.09±0.82^{a*}	0.47±0.04^{ab*}	105.65±16.59^{a*}
February	119.61±1.43^{bcd*}	56.87±2.18^{c}	11.91±0.95^{bcd*}	7.55±0.27^{b*}	1.03±0.51^{c*}	196.96±4.33^{cd*}
Correlation	No correlation					

* Less than the maximum amount at $P < 0.05$

Means not sharing a common superscript letter in the same column of one province are significantly different ($P < 0.05$) as determined by Duncan's multiple range test.

Table 4.11 The correlation between estrogenic activity of **PM-III and PM-IV sample** determine by : (vaginal cornification assay, uterotrofic assay, uterine gland number assay and the cross section area of uterine tissue assay) and the Isoflavonoid contents in glycoside form (daidzin and genistin) and aglycoside form (daidzein and genistein) in mg/100 g powder of monthly collected from Ratchaburi since March 2005 to February 2006 (mean ± S.E.M.)

month	PM-III		PM-IV		PM-III	PM-IV
	aglycoside	Glycoside	aglycoside	glycoside	aglycoside/glycoside	
March	28.50±0.47^{g}	39.70±2.29^{b*}	14.26±0.32^{c}	69.46±15.29^{d}	0.72±0.03^{f†}	0.21±0.06^{c†}
April	10.42±0.16^{de*}	60.08±5.46^{bc*}	5.10±0.51^{a*}	32.58±5.21^{ab*}	0.17±0.01^{cd*}	0.16±0.01^{abc}
May	9.65±0.94^{d*}	54.97±10.00^{bc*}	5.62±1.78^{a*}	29.13±10.8^{ab*}	0.18±0.02^{bcd*}	0.19±0.01^{abc}
June	7.71±0.94^{bcd*}	62.96±4.16^{c*}	6.69±1.17^{abc*}	45.76±12.12^{ab*}	0.13±0.02^{ab*}	0.15±0.03^{abc}
July	13.04±1.70^{e*}	43.17±6.17^{b*}	9.39±1.31^{cd*}	48.00±8.24^{abc*}	0.30±0.03^{e*}	0.21±0.10^{bc}
August	5.41±0.56b^{ab*}	46.03±2.01^{bc*}	4.09±0.37^{a*}	34.85±6.77^{a*}	0.12±0.01^{a*}	0.12±0.02^{a*}
September	10.72±0.70^{de*}	55.21±5.56^{bc*}	4.68±1.52^{a*}	35.59±3.55^{ab*}	0.19±0.02^{cd*}	0.13±0.03^{ab*}
October	5.68±0.29^{abc*}	53.05±5.89^{bc*}	6.37±0.43^{ab*}	43.90±4.46^{ab*}	0.11±0.01^{a*}	0.12±0.02^{ab}
November	8.90±0.79^{cd*}	40.83±2.85^{b*}	7.67±0.59^{a*}	36.38±1.67^{ab*}	0.22±0.02^{d*}	0.21±0.01^{abc}
December	19.08±1.84^{f*}	58.07±7.03^{bc*}	9.16±1.91^{abc*}	45.39±4.51^{ab*}	0.33±0.03^{e*†}	0.21±0.01^{abc†}
January	3.74±0.57^{a*}	19.40±3.62^{a*}	4.56±0.84^{a*}	23.10±4.85^{a*}	0.19±0.01^{cd*}	0.20±0.02^{abc}
February	13.54±1.88^{e*}	96.50±13.93^{d}	8.57±0.24^{bc*}	68.78±3.14^{bc}	0.14±0.00^{abc*}	0.12±0.01^{a*}
Correlation	No correlation					

* Less than the maximum amount at $P < 0.05$ .Means not sharing a common superscript letter in the same column of one province are significantly different ($P < 0.05$) as determined by Duncan's multiple range test.

Table 4.12 The correlation between estrogenic activity of **PM-III sample** determine by : (vaginal cornification assay, uterotrofic assay, uterine gland number assay and the cross section area of uterine tissue assay) and the ratio of individual isoflavonoid vs. puerarin in PM-III tubers monthly collected from Ratchaburi since March 2005 to February 2006 (mean ± S.E.M.)

month	daidzin/puerarin	genistin/puerarin	Daidzein/puerarin	genistein/puerarin
March	$0.93\pm0.05^{f*}$	0.30 ± 0.00^{bc}	0.68 ± 0.13^{d}	0.20 ± 0.10^{d}
April	$0.64\pm0.16^{ef*}$	0.26 ± 0.11^{c}	$0.15\pm0.05^{c*}$	$0.01\pm0.01^{ab*}$
May	$0.60\pm0.06^{cd*}$	$0.15\pm0.01^{a*}$	$0.13\pm0.00^{b*}$	$0.00\pm0.00^{a*}$
June	$0.59\pm0.30^{bcd*}$	$0.27\pm0.13^{ab*}$	$0.10\pm0.08^{ab*}$	$0.01\pm0.00^{ab*}$
July	$0.18\pm0.06^{a*}$	$0.15\pm0.02^{a*}$	$0.09\pm0.01^{ab*}$	$0.01\pm0.00^{ab*}$
August	$0.63\pm0.06^{de*}$	$0.19\pm0.02^{ab*}$	$0.08\pm0.01^{ab*}$	$0.01\pm0.00^{ab*}$
September	$0.45\pm0.05^{abcd*}$	$0.14\pm0.02^{a*}$	$0.10\pm0.01^{b*}$	$0.01\pm0.00^{ab*}$
October	$0.42\pm0.01^{abcd*}$	$0.13\pm0.01^{a*}$	$0.04\pm0.00^{a*}$	$0.02\pm0.00^{bc*}$
November	$0.30\pm0.06^{abc*}$	$0.17\pm0.06^{ab*}$	$0.09\pm0.03^{b*}$	$0.02\pm0.00^{abc*}$
December	$0.46\pm0.06^{bcd*}$	0.28 ± 0.06^{bc}	$0.22\pm0.02^{c*}$	$0.03\pm0.01^{c*}$
January	$0.30\pm0.03^{ab*}$	$0.20\pm0.01^{ab*}$	$0.09\pm0.00^{ab*}$	$0.01\pm0.00^{ab*}$
February	1.29 ± 0.23^{g}	0.31 ± 0.05^{bc}	$0.21\pm0.04^{c*}$	$0.01\pm0.00^{ab*}$
Correlation	No correlation			

*Less than the maximum amount at $P < 0.05$.

Means not sharing a common superscript letter in the same column of one province are significantly different ($P < 0.05$) as determined by Duncan's multiple range test.

Table 4.13 The correlation between estrogenic activity of **PM-IV sample** determine by : (vaginal cornification assay, uterotrofic assay, uterine gland number assay and the cross section area of uterine tissue assay) and the ratio of individual isoflavonoid vs. puerarin in PM-III tubers monthly collected from Ratchaburi since March 2005 to February 2006 (mean ± S.E.M.)

month	daidzin/puerarin	genistin/puerarin	daidzein/puerarin	genistein/puerarin
March	$0.26\pm0.07^{ab*}$	$0.11\pm0.03^{ab*}$	$0.08\pm0.00^{d*}$	$0.00\pm0.00^{a*}$
April	0.49 ± 0.05^{a}	$0.10\pm0.02^{ab*}$	$0.08\pm0.01^{d*}$	0.01 ± 0.01^{de}
May	$0.26\pm0.01^{ab*}$	$0.04\pm0.02^{a*}$	$0.06\pm0.01^{bcd*}$	$0.00\pm0.00^{a*}$
June	$0.32\pm0.05^{a*}$	$0.12\pm0.02^{ab*}$	$0.06\pm0.01^{bcd*}$	$0.00\pm0.00^{a*}$
July	0.47 ± 0.11^{b}	0.28 ± 0.05^{c}	0.12 ± 0.02^{e}	$0.01\pm0.00^{bcd*}$
August	$0.00\pm0.06^{a*}$	$0.08\pm0.01^{ab*}$	$0.04\pm0.01^{ab*}$	$0.00\pm0.00^{ab*}$
September	0.30 ± 0.05^{a}	$0.08\pm0.01^{a*}$	$0.04\pm0.01^{abc*}$	$0.00\pm0.00^{bc*}$
October	$0.33\pm0.00^{a*}$	$0.06\pm0.00^{a*}$	$0.05\pm0.00^{a*}$	0.01 ± 0.00^{de}
November	$0.26\pm0.02^{ab*}$	$0.13\pm0.04^{b*}$	$0.07\pm0.01^{cd*}$	0.02 ± 0.00^{e}
December	$0.17\pm0.01^{a*}$	$0.09\pm0.00^{ab*}$	$0.04\pm0.00^{ab*}$	$0.01\pm0.00^{cd*}$
January	$0.19\pm0.01^{ab*}$	$0.10\pm0.01^{ab*}$	$0.05\pm0.01^{abc*}$	$0.01\pm0.00^{cd*}$
February	0.48 ± 0.01^{a}	$0.10\pm0.01^{ab*}$	$0.06\pm0.00^{bcd*}$	$0.01\pm0.00^{abc*}$
Correlation	No correlation			

*Less than the maximum amount at $P < 0.05$.

Means not sharing a common superscript letter in the same column of one province are significantly different ($P < 0.05$) as determined by Duncan's multiple range test.