CHAPTER IV

RESULTS

1. Effects of *Pueraria mirifica* on vaginal cornification in ovariectomized rats.

After 2 weeks of ovariectomy, the ovariectomized rats exhibited only leucocyte cells (L-type). It was confirmed by the fact that the ovaries were completely removed and no endrogenous ovarian estrogens was produced.

1.Control group.

1.1 Negative control; The administration of distilled water did not influence the vaginal epithelium, only L-type cells were found (Figure 4-1).

1.2 Positive control; The subcutaneous injection of 200 μg/100g BW/day of 17β-estradiol 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 4-2).

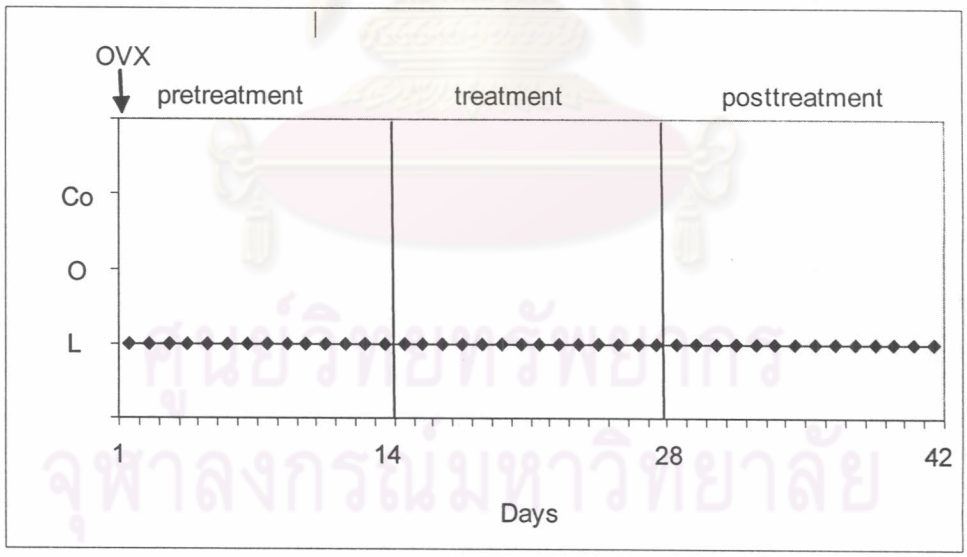

Figure 4-1. Changes of vaginal epithelium cells in rats treated with distilled water (Co = cornified cells, O= nucleated cells , L = leucocyte cells, OVX= ovariectomy).

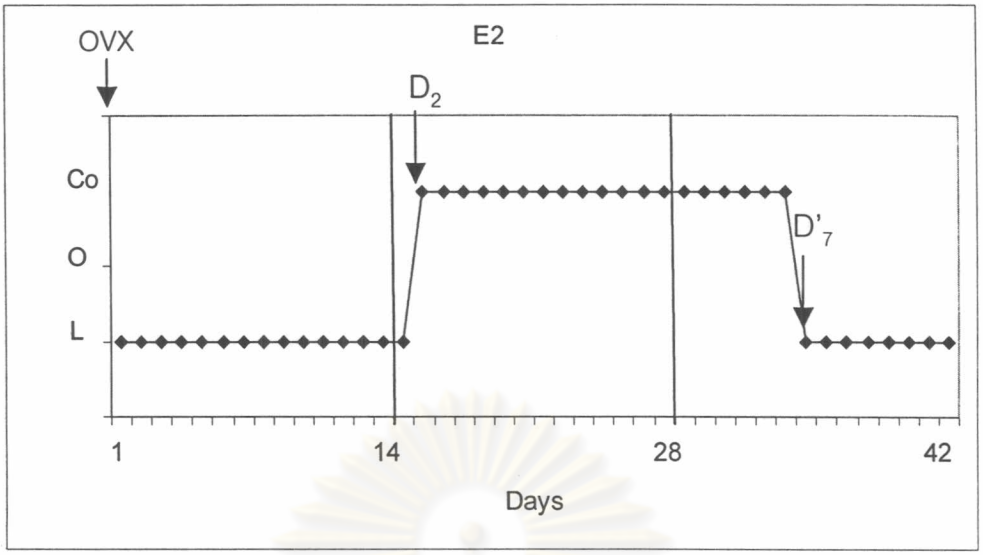

Figure 4-2. Changes of vaginal epithelium cells in rats treated with 200 μg/kg/day of 17β-estradiol (Co= cornified cells, O= nucleated cells, L= leucocyte cells, D=Day with appearance of cornified cells, D'= Day with appearance of leucocyte cells, OVX= ovariectomy).

2. *P. mirifica* treatment group

The results are presented in Figure 4-3 to 4-27. It was described according to the geographical distribution of the collected sites as follows;

2.1 The northern part of Thailand

By the rat vaginal cornification assay of PM sample collected from **Chiang Rai**, the dosage 10 mg/kg BW/day did not have any effect on vaginal epithelium, only L-type cells was found. At the dosage of 100 mg/kg BW/day, the cell type was changed from L to Co within 5 days of the 14-day treatment period and returned to L-type cells within 5 days after cessation of the treatment. However, the cell type was changed from L-type cells to Co-type cells within 3 days of the 14-day treatment period of 1,000 mg/kg BW/day and recovered within 6 days after cessation of the treatment (Figure 4-3).

By the rat vaginal cornification assay of PM sample collected from **Mae Hong Son**, the dosage 10 mg/kg BW/day did not have any effect on vaginal epithelium, only L-type cells was found. At the dosage of 100 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 returned to L

within 3 days after cessation of the treatment. However, the cell type was changed from L to Co within 4 days of the 14-day treatment period of 1,000 mg/kg BW/day and recovered within 5 days after cessation of the treatment (Figure 4-4).

By the rat vaginal cornification assay of PM sample collected from **Phayoa**, the dosage of 10 mg/kg BW/day did not have any effect on vaginal epithelium, only L-type cells was found. At the dosage of 100 mg/kg BW/day, the cell type was changed from L-type cells to O-type cells within 5 days of the 14-day treatment period and returned to L-type cells within 3 days after cessation of the treatment. However, the cell type was changed from L to Co within 5 days of the 14-day treatment period of the dosage of 1,000 mg/kg BW/day and recovered within 4 days after cessation of the treatment (Figure 4-5).

By the rat vaginal cornification assay of the treatment with PM sample collected from **Nan**, the dosage of 10 mg/kg BW/day did not have any effect on vaginal epithelium, only L-type cells was found. At the dosage of 100 mg/kg BW/day, the cell type was changed from L-type cells to O-type cells within 5 days of the 14-day treatment period and returned to L-type cells within 1 days after cessation of the treatment. However, the cell type was changed from L to Co within 4 days of the 14-day treatment period of the dosage of 1,000 mg/kg BW/day and recovered within 5 days after cessation of the treatment (Figure 4-6).

By the rat vaginal cornification assay of PM sample collected from **Lumpang**, the dosage of 10 mg/kg BW/day did not have any effect on vaginal epithelium, only L-type cells was found. At the dosage of 100 mg/kg BW/day, the cell type was changed from L-type cells to O-type cells within 5 days of the 14-day treatment period and returned to L-type cells within 4 days after cessation of the treatment. However, the cell type was changed from L to Co within 5 days of the 14-day treatment period of 1,000 mg/kg BW/day and recovered within 5 days after cessation of the treatment (Figure 4-7).

By the rat vaginal cornification assay of PM sample collected from **Phrae**, the dosage of 10 mg/kg BW/day did not have any effect on vaginal epithelium, only L-type cells was found. At the dosage of 100 mg/kg BW/day, the cell type was changed from L-type cells to O-type cells within 4 days of the 14-day treatment period and returned to L-type cells within 5 days after cessation of the treatment. However, the cell type was changed from L to Co within 4 days of the 14-day treatment period of the dosage of 1,000 mg/kg BW/day and recovered within 5 days after cessation of the treatment (Figure 4-8).

By the rat vaginal cornification assay of PM sample collected from **Lumphun**, the dosage 10 mg/kg BW/day did not have any effect on vaginal epithelium, only L-type cells was found. At the dosage of 100 mg/kg BW/day, the cell type was changed from L-type cells to O-type cells within 5 days of the 14-day treatment period and returned to L-type cells within 4 days after cessation of the treatment. However, the cell type was changed from L to Co within 5 days of the 14-day treatment period of the dosage of 1,000 mg/kg BW/day and recovered within 5 days after cessation of the treatment (Figure 4-9).

By the rat vaginal cornification assay of PM sample collected from **Uttharadith**, the dosage of 10 mg/kg BW/day did not have any effect on vaginal epithelium, only L-type cells was found. At the dosage of 100 mg/kg BW/day, the cell type was changed from L-type cells to O-type cells within 6 days of the 14-day treatment period and returned to L-type cells within 2 days after cessation of the treatment. However, the cell type was changed from L to Co within 8 days of the 14-day treatment period of the dosage of 1,000 mg/kg BW/day and recovered within 3 days after cessation of the treatment (Figure 4-10).

By the rat vaginal cornification assay of PM sample collected from **Sukhothai**, the dosage of 10 mg/kg BW/day did not have any effect on vaginal epithelium, only L-type cells was found. At the dosage of 100 mg/kg BW/day, the cell type was changed from L-type cells to Co-type cells within 5 days of the 14-day treatment period and returned to L-type cells within 4 days after cessation of the treatment. However, the cell type was changed from L to Co within 4 days of the 14-day treatment period of the dosage of 1,000 mg/kg BW/day and recovered within 6 days after cessation of the treatment (Figure 4-11).

By the rat vaginal cornification assay of PM sample collected from **Tak**, the dosage of10 mg/kg BW/day did not have any effect on vaginal epithelium, only L-type cells was found. At the dosage of 100 mg/kg BW/day, the cell type was changed from L-type cells to O-type cells within 4 days of the 14-day treatment period and returned to L-type cells within 4 days after cessation of the treatment. However, the cell type was changed from L to Co within 4 days of the 14-day treatment period of the dosage of 1,000 mg/kg BW/day and recovered within 7 days after cessation of the treatment (Figure 4-12).

By the rat vaginal cornification assay of PM sample collected from **Phitsanulok**, the dosage of 10 mg/kg BW/day exhibited nucleated cell within 6 days of the 14-day treatment period which changed to L-type cells within 3 days after the cessation of treatment. At the

dosage of 100 mg/kg BW/day, the cell type was changed from L-type cells to Co-type cells within 6 days of the 14-day treatment period and returned to L-type cells within 4 days after cessation of the treatment. However, the cell type was changed from L to Co within 2 days of the 14-day treatment period of the dosage of 1,000 mg/kg BW/day and recovered within 6 days after cessation of the treatment (Figure 4-13).

By the rat vaginal cornification assay of PM sample collected from **Phetchabun**, the lowest dose of PM did not have any effect on vaginal epithelium, only L-type cells was found. At the dosage of 100 mg/kg BW/day, the cell type was changed from L-type cells to Co-type cells within 6 days of the 14-day treatment period and returned to L-type cells within 2 days after cessation of the treatment. However, the cell type was changed from L to Co within 3 days of the 14-day treatment period of the dosage of 1,000 mg/kg BW/day and recovered within 4 days after cessation of the treatment (Figure 4-14).

By the rat vaginal cornification assay of PM sample collected from **Kampaeng Phet**, the dosage of 10 mg/kg BW/day exhibited only L-type cells. At the dosage of100 mg/kg BW/day, the cell type was changed from L-type cells to Co-type cells within 6 days of the 14-day treatment period and returned to L-type cells within 4 days after cessation of the treatment. However, the cell type was changed from L to Co within 4 days of the 14-day treatment period of 1,000 mg/kg BW/day and recovered within 6 days after cessation of the treatment (Figure 4-15).

By the rat vaginal cornification assay of PM sample collected from **Nakorn Sawan**, the dosage of 10 mg/kg BW/day exhibited nucleated cell within 6 days of the 14-day treatment period which changed to L-type cells within 2 days after the cessation of treatment. At the dosage of 100 mg/kg BW/day, the cell type was changed from L-type cells to Co-type cells within 5 days of the 14-day treatment period and returned to L-type cells within 2 days after cessation of the treatment. However, the cell type was changed from L-type cells to Co-type cells within 3 days of the 14-day treatment period of the dosage of 1,000 mg/kg BW/day and recovered within 6 days after cessation of the treatment (Figure 4-16).

By the rat vaginal cornification assay of PM sample collected from **Uthai thani**, the dosage of 10 mg/kg BW/day exhibited only L-type cells. At the dosage of 100 mg/kg BW/day, the cell type was changed from L-type cells to O-type cells within 5 days of the 14-

day treatment period and returned to L-type cells within 4 days after cessation of the treatment. However, the cell type was changed from L to Co within 4 days of the 14-day treatment period of the dosage of 1,000 mg/kg BW/day and recovered within 5 days after cessation of the treatment (Figure 4-17).

2.2 The north eastern part of Thailand.

By the rat vaginal cornification assay of PM sample collected from **Sakon Nakhon**, the dosage of 10 mg/kg BW/day did not have any effect on vaginal epithelium, only L-type cells was found. At the dosage of 100 mg/kg BW/day, the cell type was changed from L-type cells to Co-type cells within 7 days of the 14-day treatment period and returned to L-type cells within 1 days after cessation of the treatment However, the cell type was changed from L to Co within 6 days of the 14-day treatment period of the dosage of 1,000 mg/kg BW/day and recovered within 3 days after cessation of the treatment (Figure 4-18).

By the rat vaginal cornification assay of PM sample collected from **Nong Bua Lam Phu**, the dosage of 10 mg/kg BW/day did not have any effect on vaginal epithelium, only L-type cells was found. At the dosage of 100 mg/kg BW/day, the cell type was changed from L-type cells to O-type cells within 4 days of the 14-day treatment period and returned to L-type cells within 5 days after cessation of the treatment. However, the cell type was changed from L to Co within 5 days of the 14-day treatment period of the dosage of 1,000 mg/kg BW/day and recovered within 4 days after cessation of the treatment (Figure 4-19).

By the rat vaginal cornification assay of PM sample collected from **Chaiyaphum**, the dosage of 10 mg/kg BW/day exhibited only L-type cells. At the dosage of100 mg/kg BW/day, the cell type was changed from L-type cells to Co-type cells within 5 days of the 14-day treatment period and returned to L-type cells within 4 days after cessation of the treatment. However, the cell type was changed from L-type cells to Co-type cells within 5 days of the 14-day treatment period of the dosage of 1,000 mg/kg BW/day and recovered within 5 days after cessation of the treatment (Figure 4-20).

By the rat vaginal cornification assay of PM sample collected from **Nakorn Ratchasima**, the dosage of 10 mg/kg BW/day exhibited nucleated cell within 5 days of the 14-day treatment period which changed to L-type cells within 4 days after the cessation of treatment. At the dosage of100 mg/kg BW/day, the cell type was changed from L-type cells to Co-type cells within 6 days of the 14-day treatment period and returned to L-type cells

within 6 days after cessation of the treatment. However, the cell type was changed from L to Co within 3 days of the 14-day treatment period of the dosage of 1,000 mg/kg BW/day and recovered within 6 days after cessation of the treatment (Figure 4-21).

2.3 The central part of Thailand.

By the rat vaginal cornification assay of PM sample collected from **Lop Buri**, the dosage 10 mg/kg BW/day did not have any effect on vaginal epithelium, only L-type cells was found. At the dosage of100 mg/kg BW/day, the cell type was changed from L-type cells to O-type cells within 4 days of the 14-day treatment period and returned to L-type cells within 5 days after cessation of the treatment. However, the cell type was changed from L to Co within 4 days of the 14-day treatment period of 1,000 mg/kg BW/day and recovered within 3 days after cessation of the treatment (Figure 4-22).

By the rat vaginal cornification assay of PM sample collected from **Kanchanaburi**, the dosage of 10 mg/kg BW/day exhibited nucleated cell within 4 days of the 14-day treatment period which changed to L-type cells within 4 days after the cessation of treatment. At the dosage of 100 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 returned to L-type cells within 7days after cessation of the treatment. However, the cell type was changed from L to Co within 2 days of the 14-day treatment period of the dosage of 1,000 mg/kg BW/day and recovered within 6 days after cessation of the treatment (Figure 4-23).

By the rat vaginal cornification assay of PM sample collected from **Phrachin Buri**, the dosage 10 mg/kg BW/day did not have any effect on vaginal epithelium, only L-type cells was found. At the dosage of 100 mg/kg BW/day, the cell type was changed from L-type cells to Co-type cells within 6 days of the 14-day treatment period and returned to L-type cells within 2 days after cessation of the treatment. However, the cell type was changed from L to Co within 4 days of the 14-day treatment period of the dosage of 1,000 mg/kg BW/day and recovered within 4 days after cessation of the treatment (Figure 4-24).

By the rat vaginal cornification assay of PM sample collected from **Ratchaburi**, the dosage of 10 mg/kg BW/day exhibited nucleated cell within 6 days of the 14-day treatment period which changed to L-type cells within 1 days after the cessation of treatment. At the dosage of 100 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 returned to L-type cells within 5 days after cessation of the treatment. However, the cell type was changed from L-type cells to Co-type cells within 2 days of the 14-day treatment period of the dosage of 1,000 mg/kg BW/day and recovered within 6 days after cessation of the treatment (Figure 4-25).

By the rat vaginal cornification assay of PM sample collected from **Phetchaburi**, the lowest dose of PM did not have any effect on vaginal epithelium, only L-type cells was found. At the dosage of 100 mg/kg BW/day, the cell type was changed from L-type cells to O-type cells within 4 days of the 14-day treatment period and returned to L-type cells within 2 days after cessation of the treatment. However, the cell type was changed from L to Co within 8 days of the 14-day treatment period of the dosage of 1,000 mg/kg BW/day and recovered within 3 days after cessation of the treatment (Figure 4-26).

2.4 The upper southern part of Thailand.

By the rat vaginal cornification assay of PM sample collected from **Chumphon**, the dosage of 10 mg/kg BW/day exhibited nucleated cell within 6 days of the 14-day treatment period which changed to L-type cells within 3 days after the cessation of treatment. At the dosage of 100 mg/kg BW/day, the cell type was changed from L-type cells to Co-type cells within 5 days of the 14-day treatment period and returned to L-type cells within 5 days after cessation of the treatment. However, the cell type was changed from L to Co within 3 days of the 14-day treatment period of the dosage of 1,000 mg/kg BW/day and recovered within 7 days after cessation of the treatment (Figure 4-27).

The northern part of Thailand

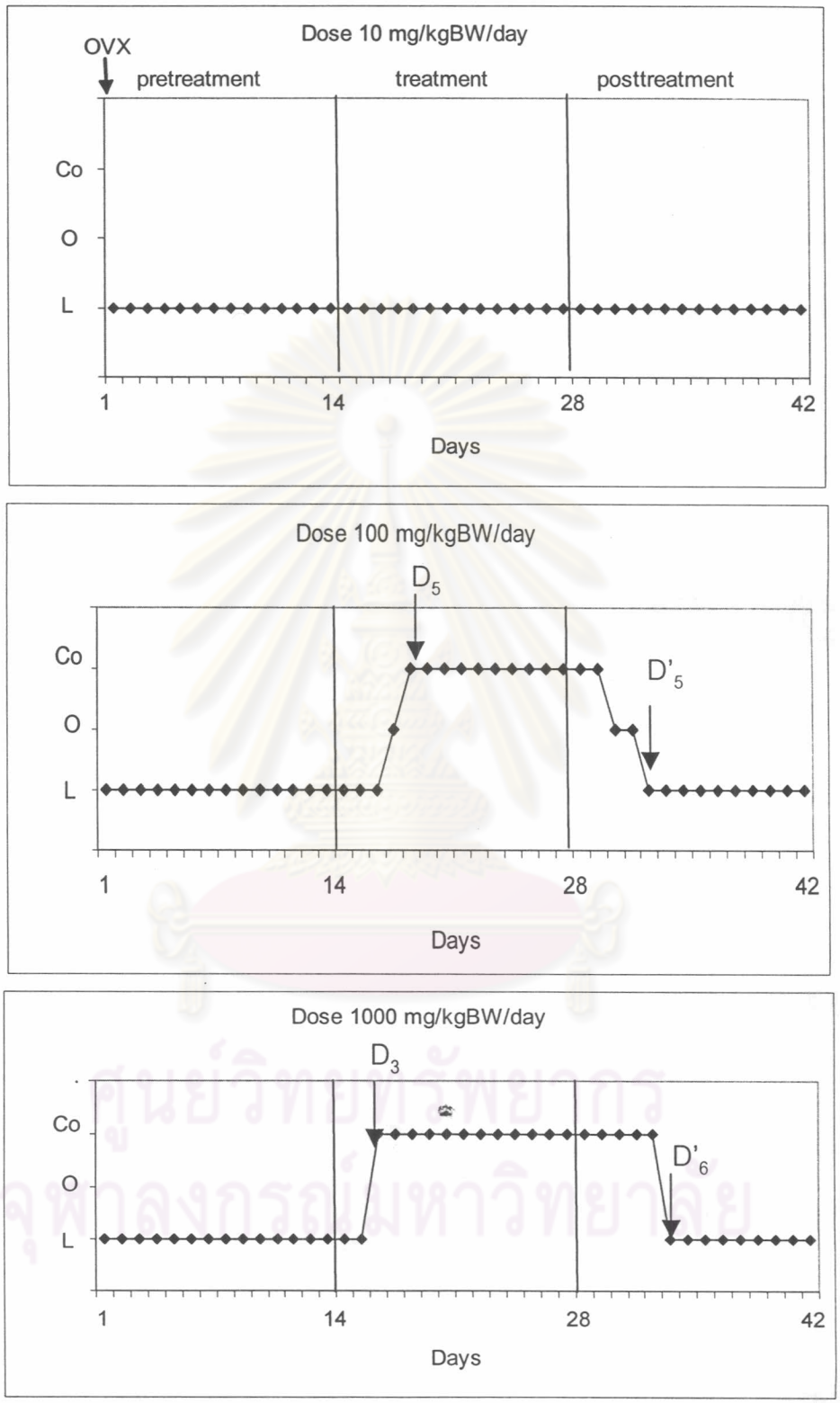

Figure 4-3. Changes of vaginal epithelium cells in rats treated with 10, 100 and 1,000 mg/kg BW/day of *P. mirifica* powder derived from tuber collected from **Chiang Rai** (Co= cornified cells, O= nucleated cells, L= leucocyte cells, D=Day with appearance of cornified cells, D'= Day with appearance of leucocyte cells).

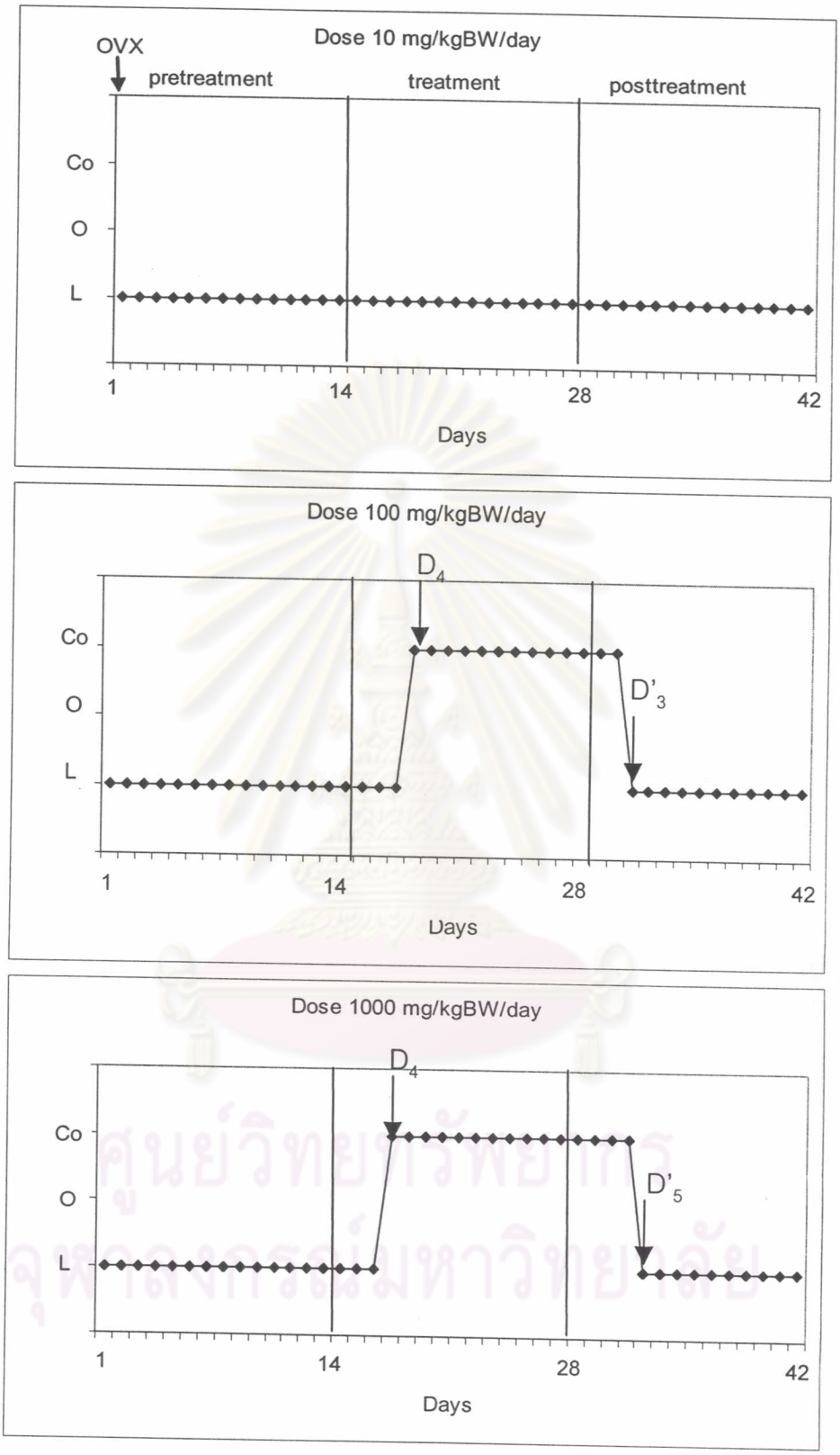

Figure 4-4. Changes of vaginal epithelium cells in rats treated with 10, 100 and 1,000 mg/kg BW/day of *P. mirifica* powder derived from tuber collected from Mae Hong Son (Co= cornified cells, O= nucleated cells, L= leucocyte cells, D=Day with appearance of cornified cells, D'= Day with appearance of leucocyte cells).

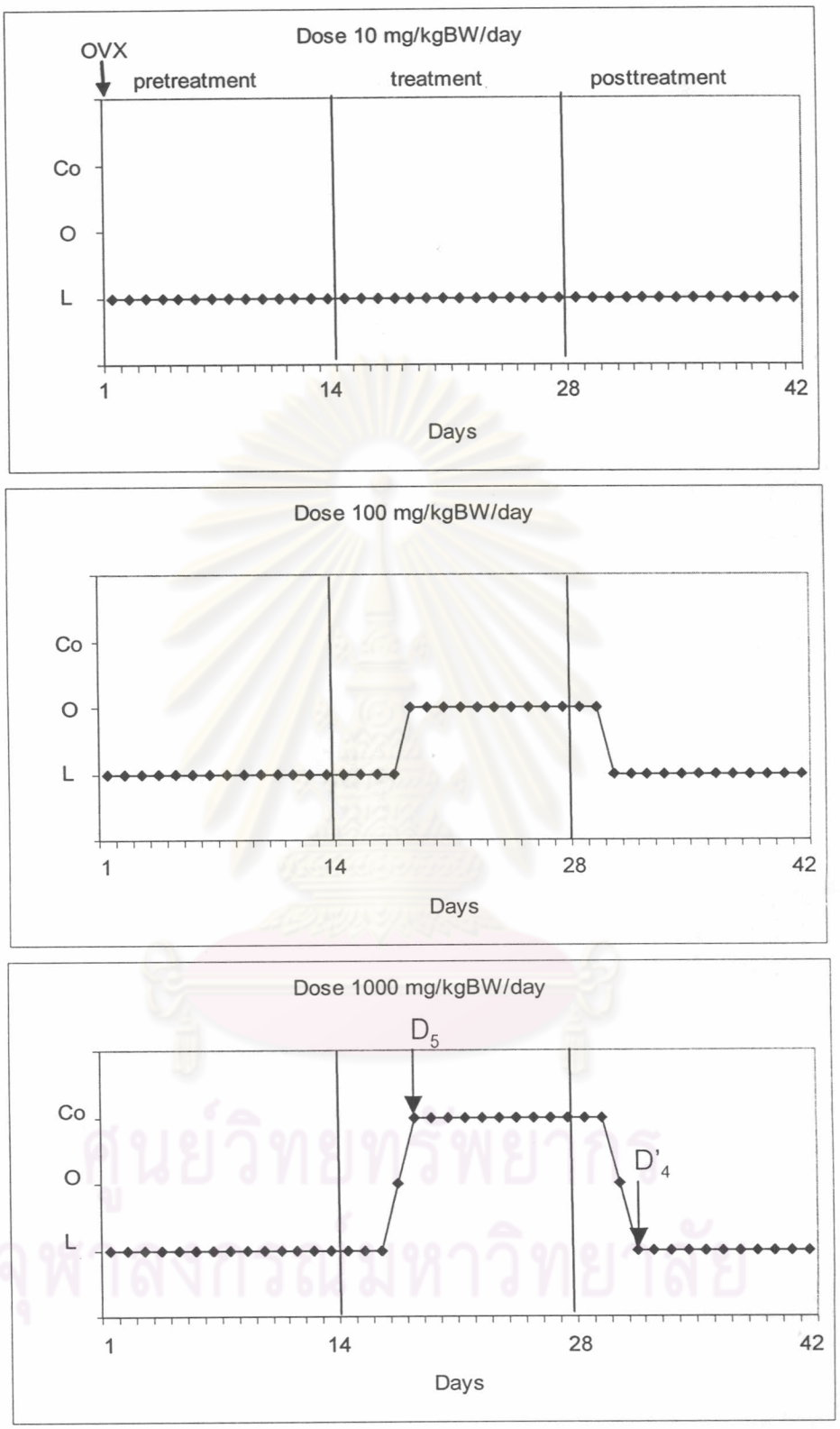

Figure 4-5. Changes of vaginal epithelium cells in rats treated with 10, 100 and 1,000 mg/kg BW/day of *P. mirifica* powder derived from tuber collected from **Phayoa** (Co= cornified cells, O= nucleated cells, L= leucocyte cells, D=Day with appearance of cornified cells, D'= Day with appearance of leucocyte cells).

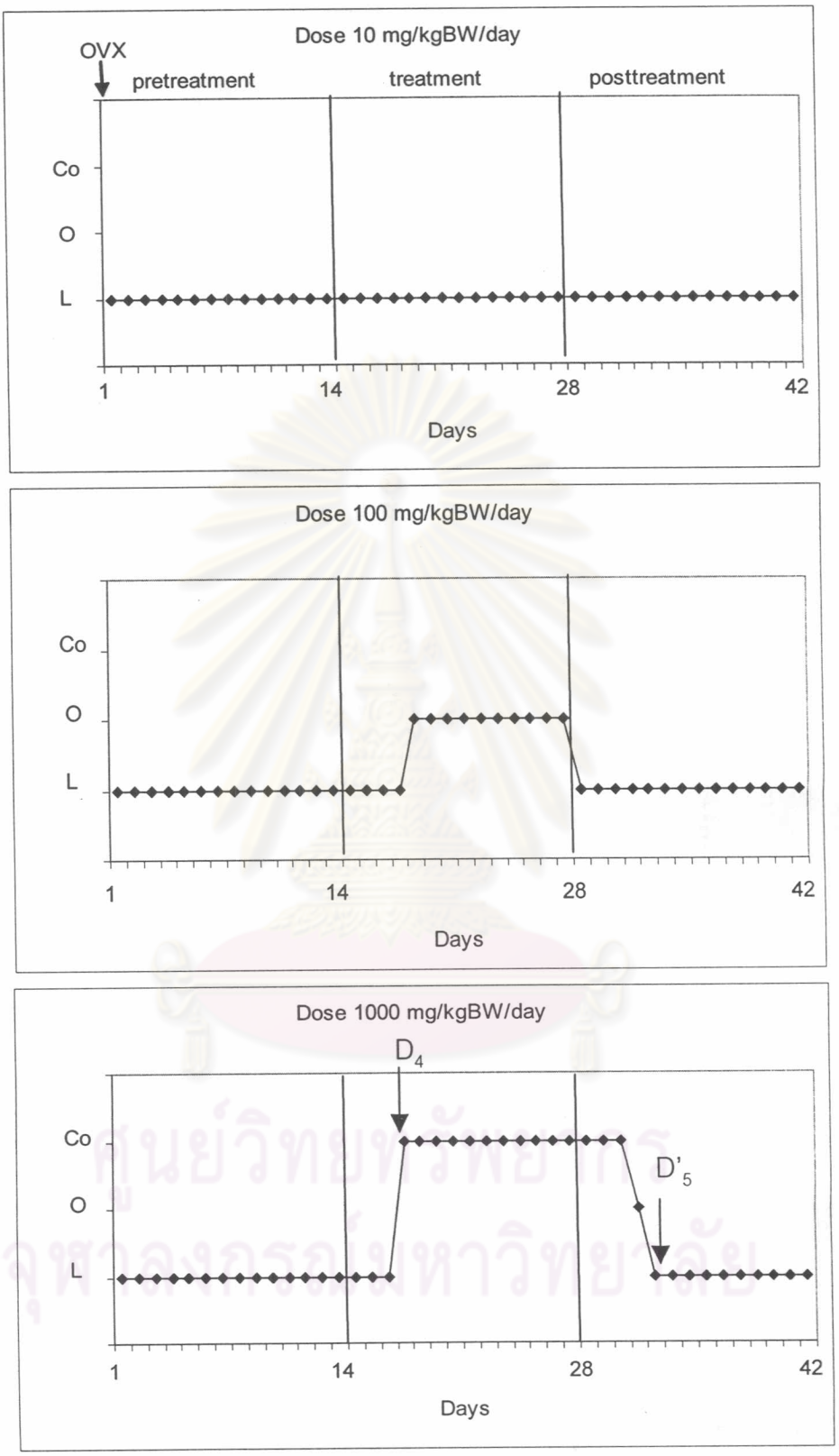

Figure 4-6. Changes of vaginal epithelium cells in rats treated with 10, 100 and 1,000 mg/kg BW/day of *P. mirifica* powder derived from the tuber collected from Nan (Co= cornified cells, O= nucleated cells, L= leucocyte cells, D=Day with appearance of cornified cells, D'= Day with appearance of leucocyte cells).

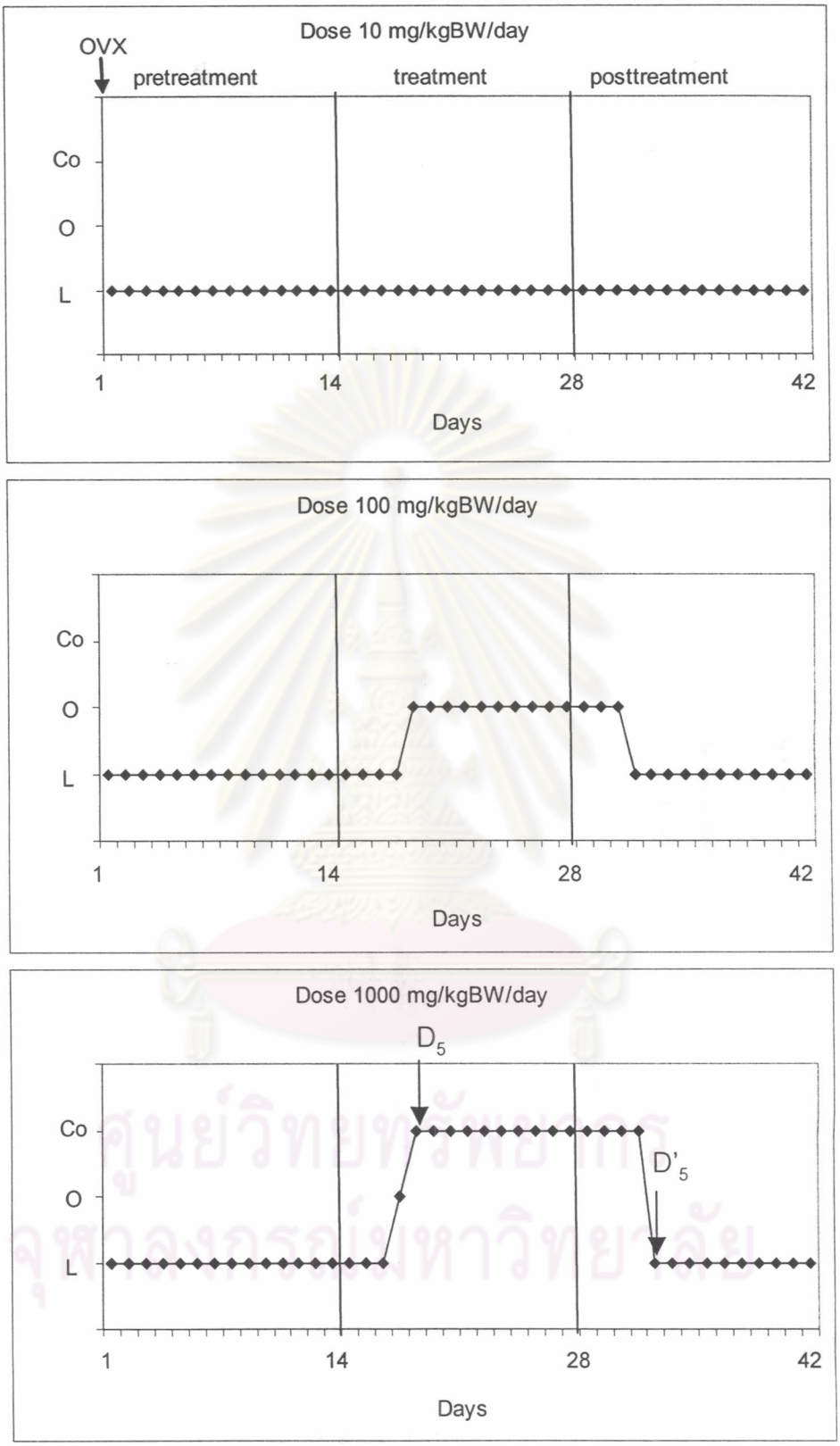

Figure 4-7. Changes of vaginal epithelium cells in rats treated with 10, 100 and 1,000 mg/kg BW/day of *P. mirifica* powder derived from the tuber collected from **Lumpang** (Co= cornified cells, O= nucleated cells, L= leucocyte cells, D=Day with appearance of cornified cells, D'= Day with appearance of leucocyte cells).

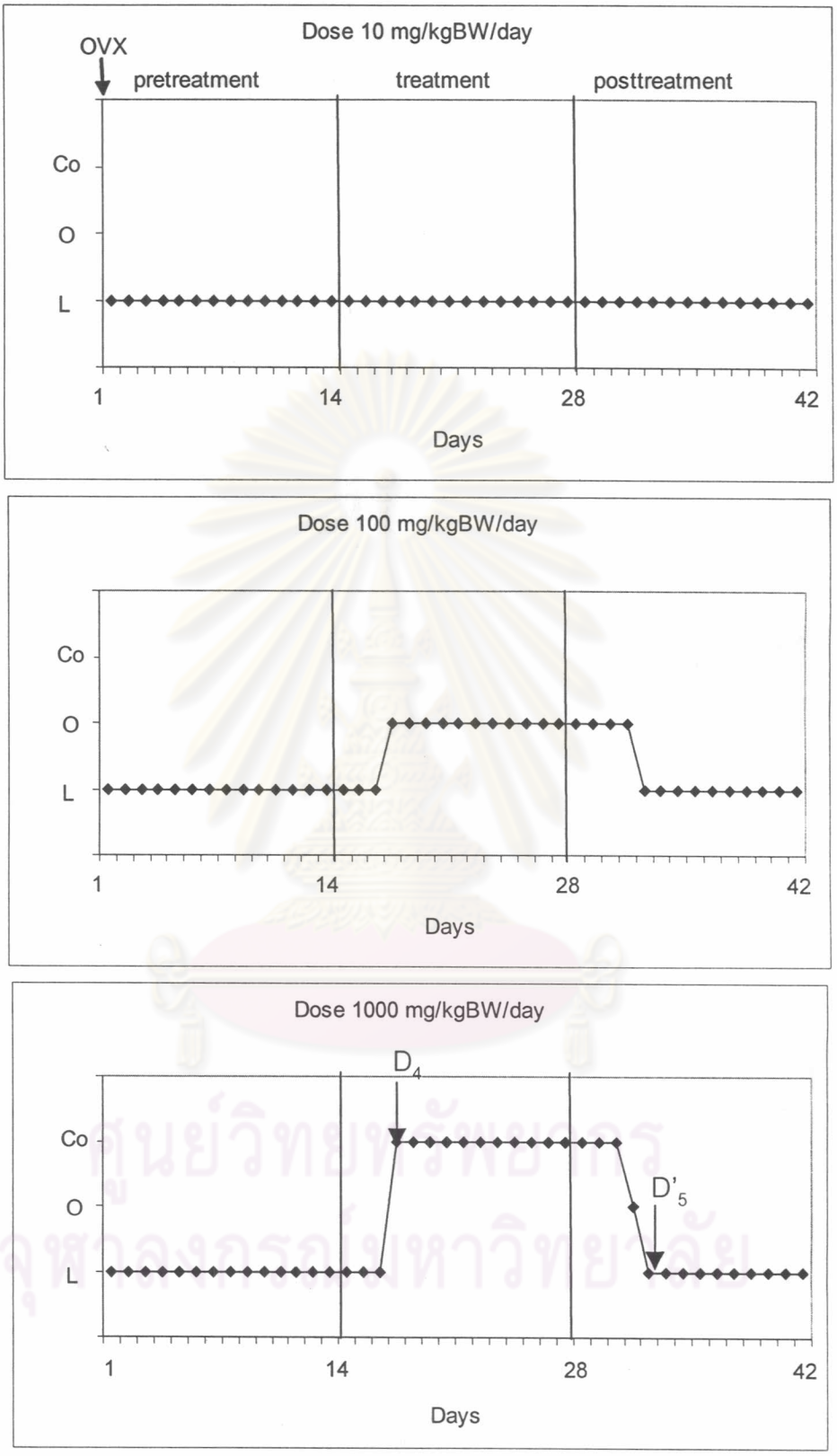

Figure 4-8. Changes of vaginal epithelium cells in rats treated with 10, 100 and 1,000 mg/kg BW/day of *P. mirifica* powder derived from the tuber collected from **Phrae** (Co= cornified cells, O= nucleated cells, L= leucocyte cells, D=Day with appearance of cornified cells, D'= Day with appearance of leucocyte cells).

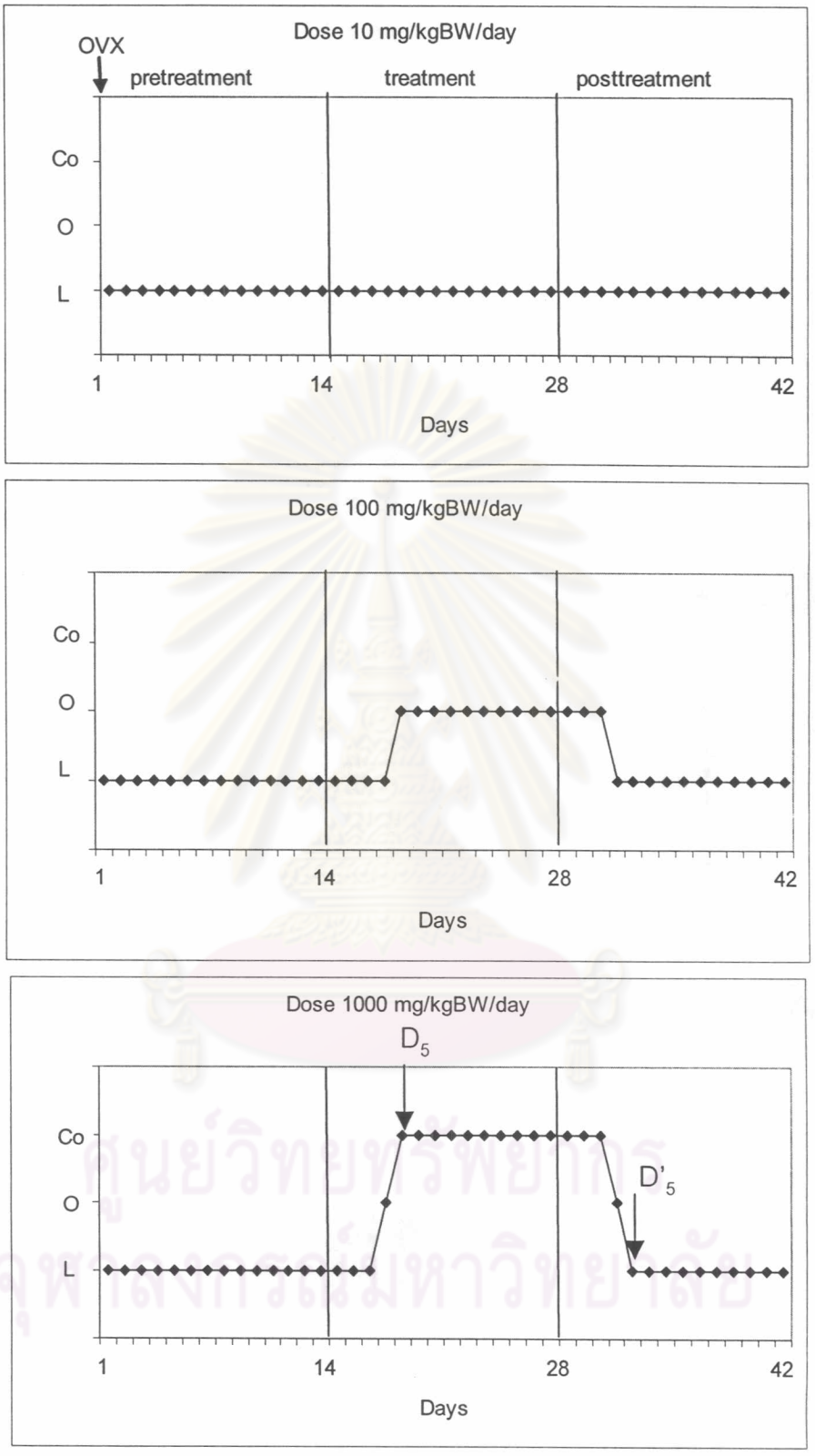

Figure 4-9. Changes of vaginal epithelium cells in rats treated with 10, 100 and 1,000 mg/kg BW/day of *P. mirifica* powder derived from the tuber collected from **Lumphun** (Co= cornified cells, O= nucleated cells, L= leucocyte cells, D=Day with appearance of cornified cells, D'= Day with appearance of leucocyte cells).

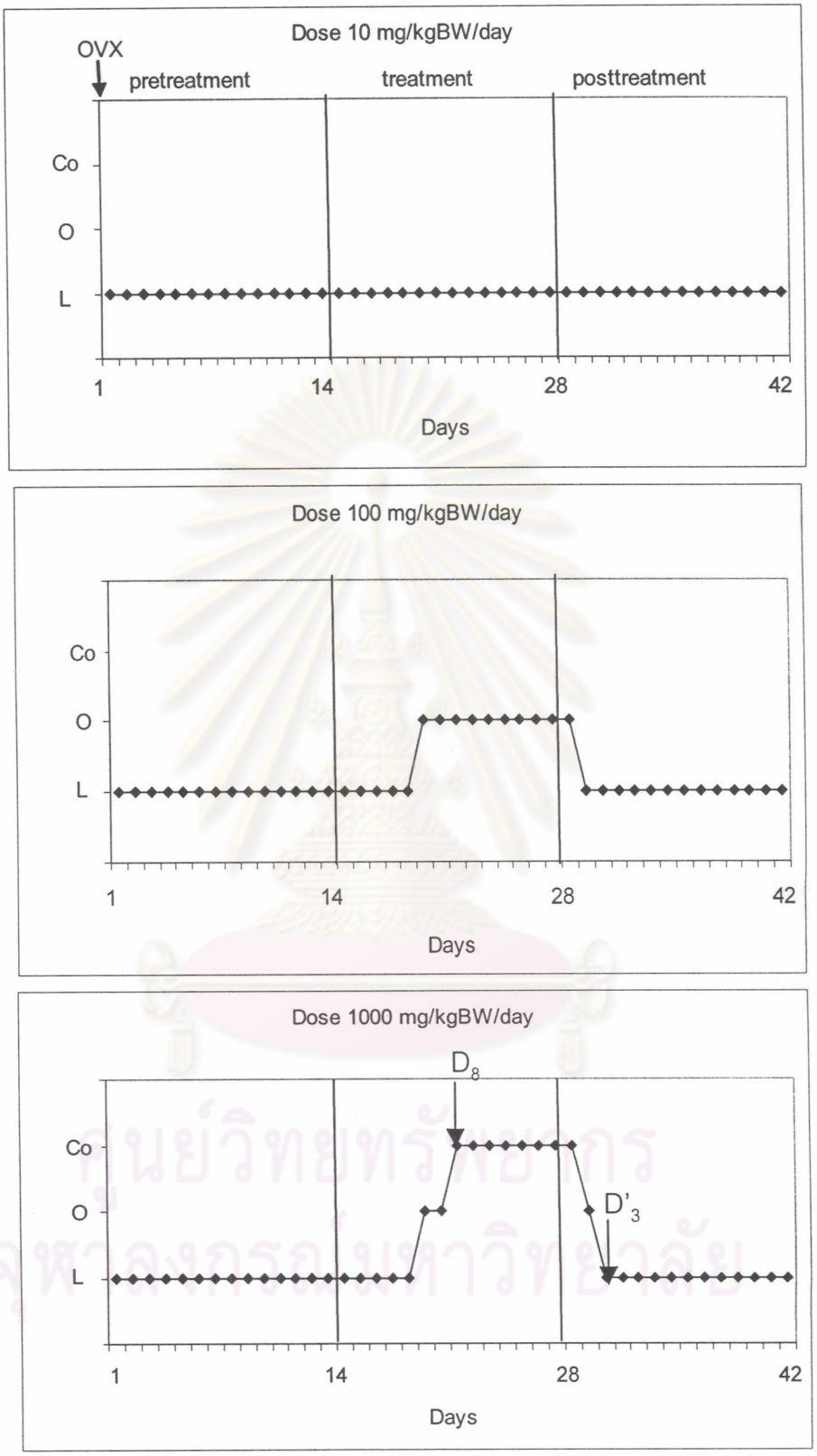

Figure 4-10. Changes of vaginal epithelium cells in rats treated with 10, 100 and 1,000 mg/kg BW/day of *P. mirifica* powder derived from the tuber collected from Uttharadith (Co= cornified cells, O= nucleated cells, L= leucocyte cells, D=Day with appearance of cornified cells, D'= Day with appearance of leucocyte cells).

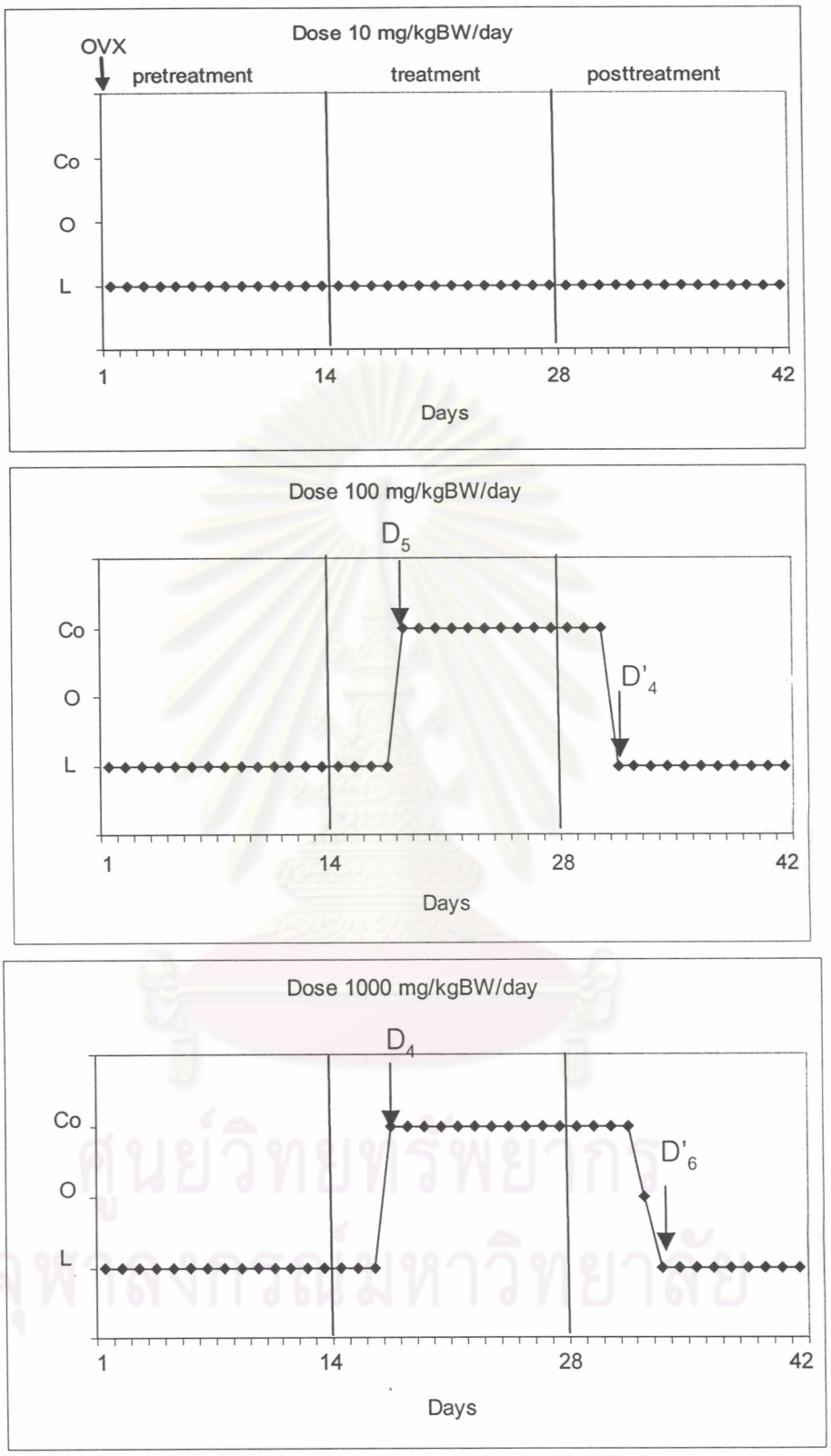

Figure 4-11. Changes of vaginal epithelium cells in rats treated with 10, 100 and 1,000 mg/kg BW/day of *P. mirifica* powder derived from the tuber collected from **Sukhothai** (Co= cornified cells, O= nucleated cells, L= leucocyte cells, D=Day with appearance of cornified cells, D'= Day with appearance of leucocyte cells).

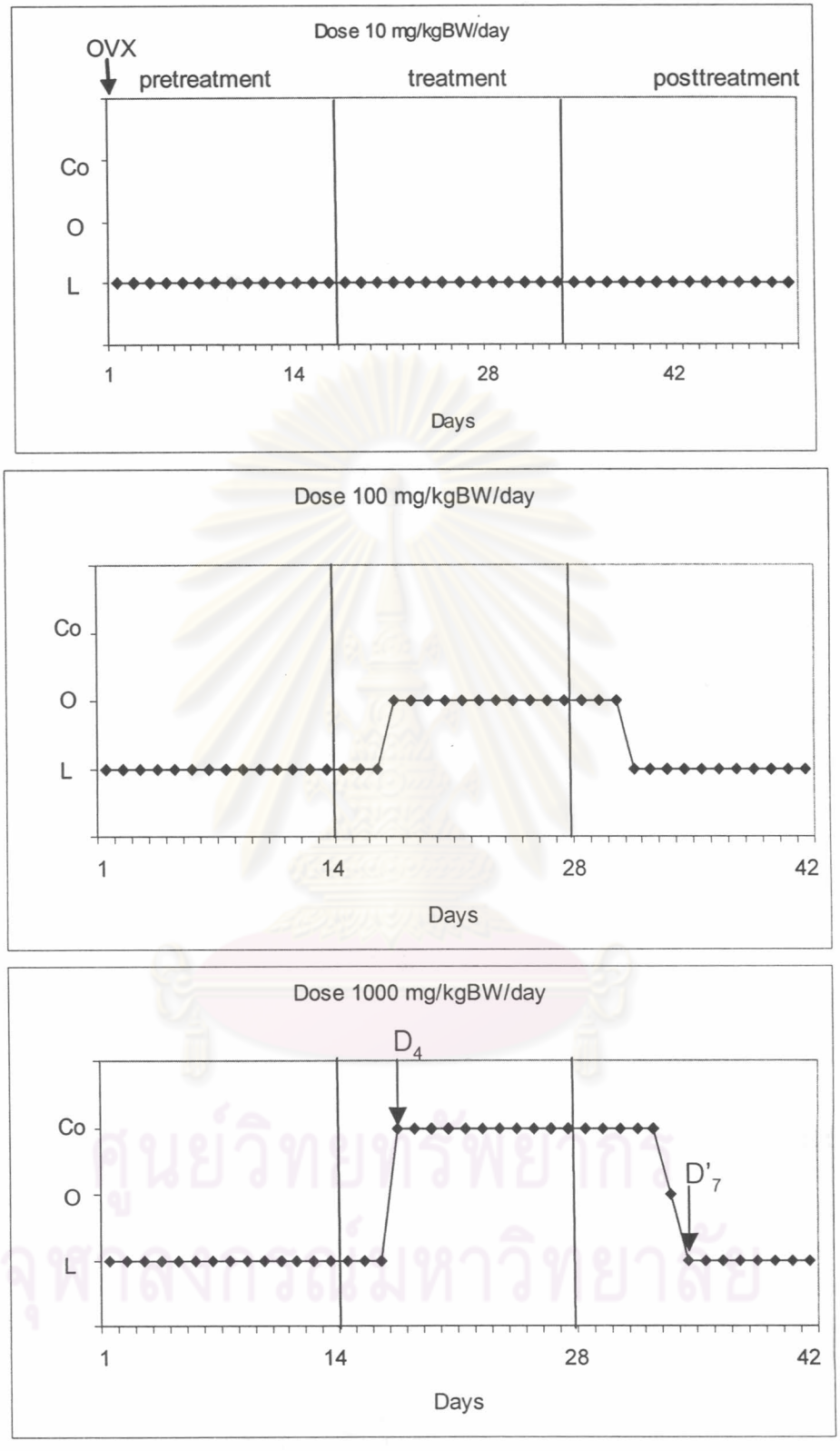

Figure 4-12. Changes of vaginal epithelium cells in rats treated with 10, 100 and 1,000 mg/kg BW/day of *P. mirifica* powder derived from the tuber collected from Tak (Co= cornified cells, O= nucleated cells, L= leucocyte cells, D=Day with appearance of cornified cells, D'= Day with appearance of leucocyte cells).

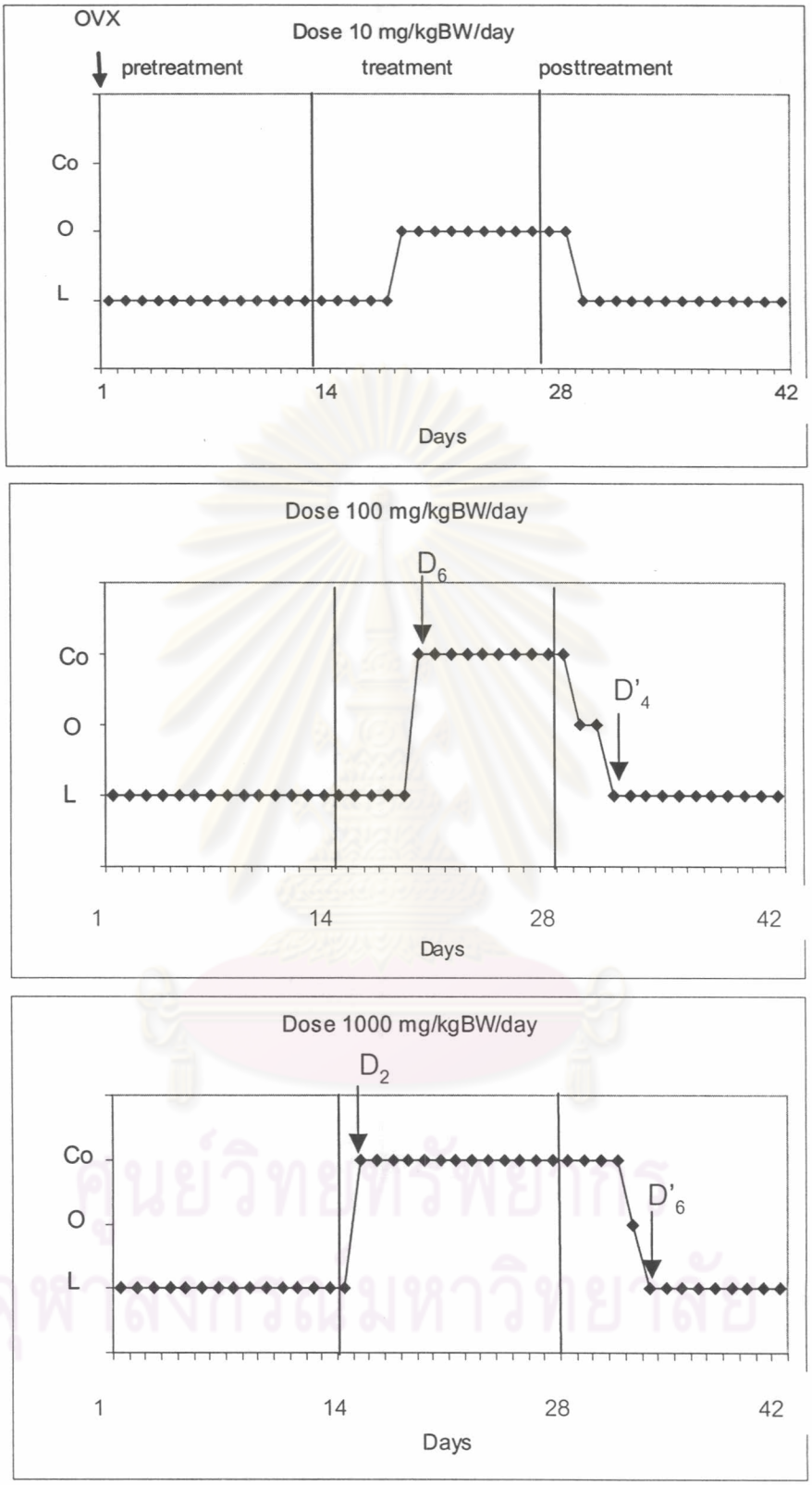

Figure 4-13. Changes of vaginal epithelium cells in rats treated with 10, 100 and 1,000 mg/kg BW/day of *P. mirifica* powder derived from the tuber collected from **Phitsanulok** (Co= cornified cells, O= nucleated cells, L= leucocyte cells, D=Day with appearance of cornified cells, D'= Day with appearance of leucocyte cells).

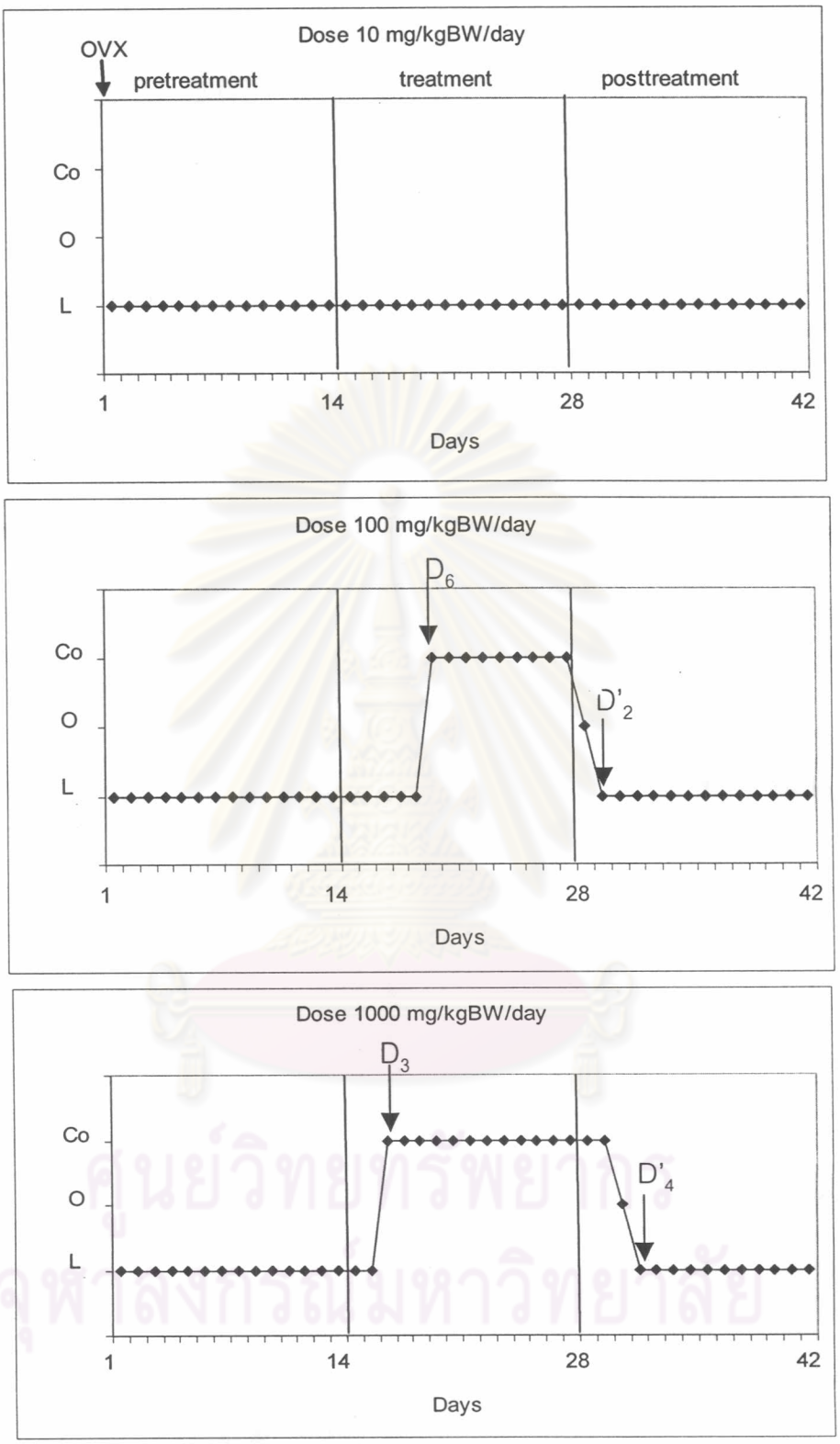

Figure 4-14. Changes of vaginal epithelium cells in rats treated with 10, 100 and 1,000 mg/kg BW/day of *P. mirifica* powder derived from the tuber collected from **Phetchabun** (Co= cornified cells, O= nucleated cells, L= leucocyte cells, D=Day with appearance of cornified cells, D'= Day with appearance of leucocyte cells).

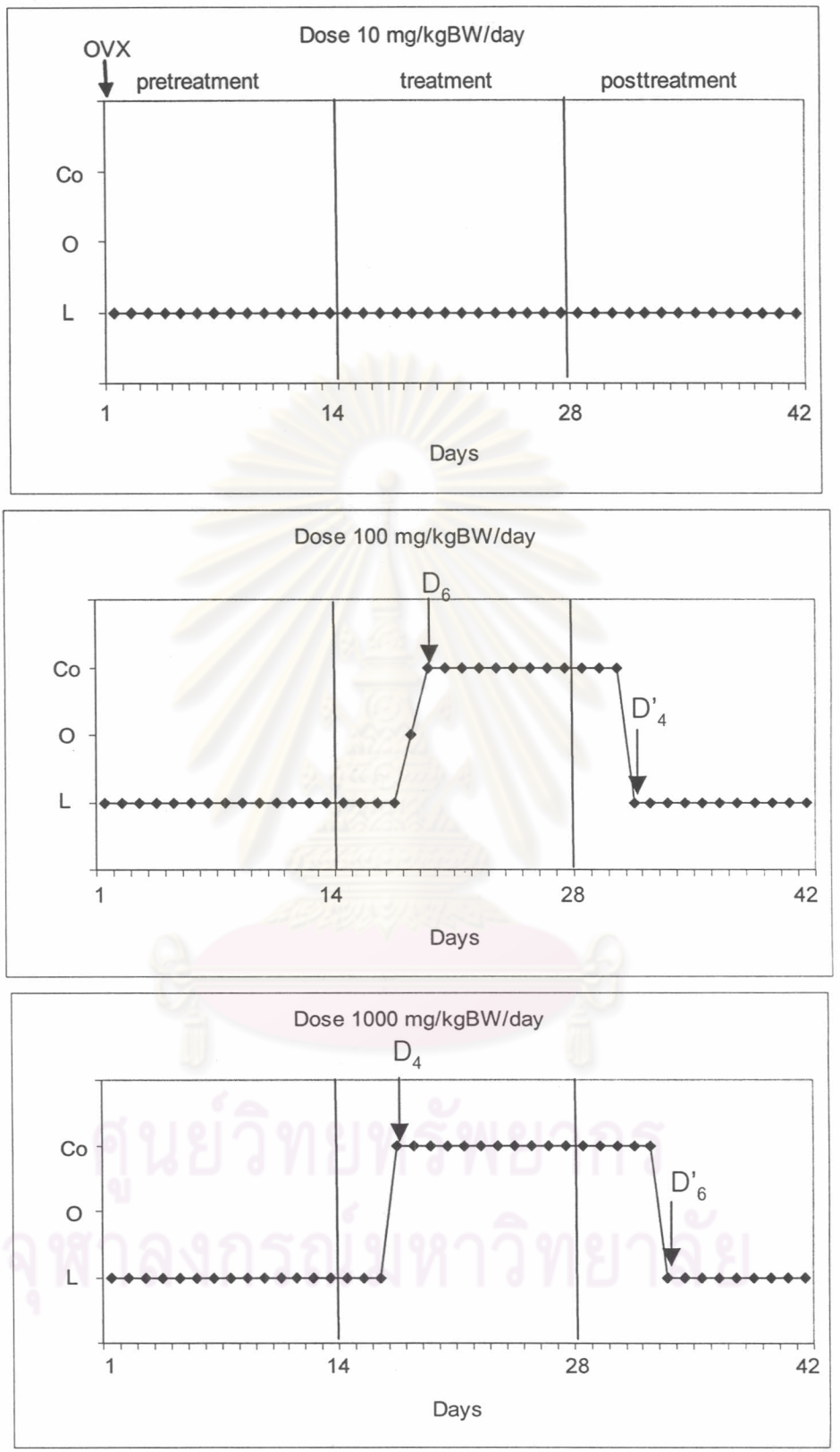

Figure 4-15. Changes of vaginal epithelium cells in rats treated with 10, 100 and 1,000 mg/kg BW/day of *P. mirifica* powder derived from the tuber collected from **Kampaeng Phet** (Co= cornified cells, O= nucleated cells, L= leucocyte cells, D=Day with appearance of cornified cells, D'= Day with appearance of leucocyte cells).

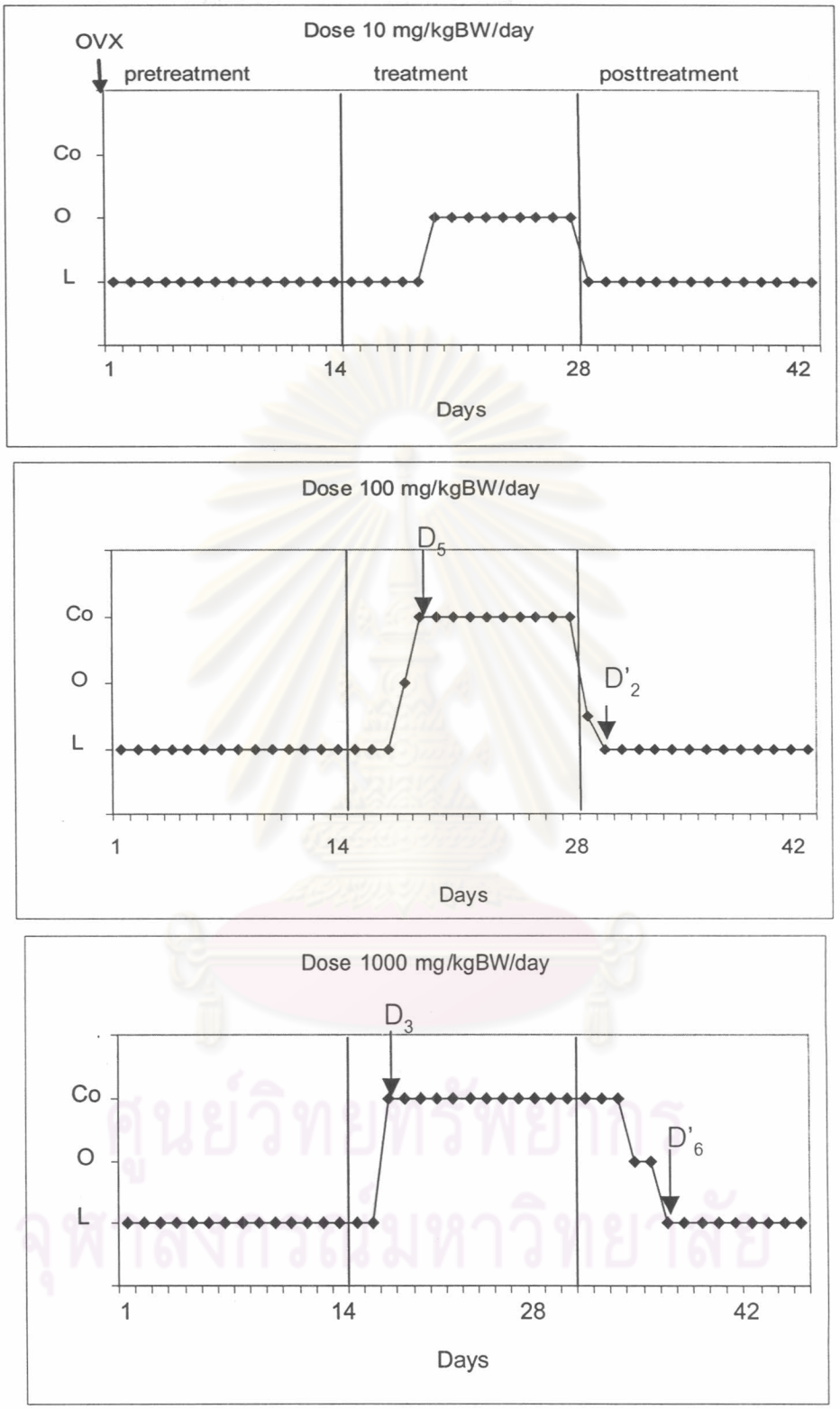

Figure 4-16. Changes of vaginal epithelium cells in rats treated with 10, 100 and 1,000 mg/kg BW/day of *P. mirifica* powder derived from the tuber collected from **Nakorn sawan** (Co= cornified cells, O= nucleated cells, L= leucocyte cells, D=Day with appearance of cornified cells, D'= Day with appearance of leucocyte cells).

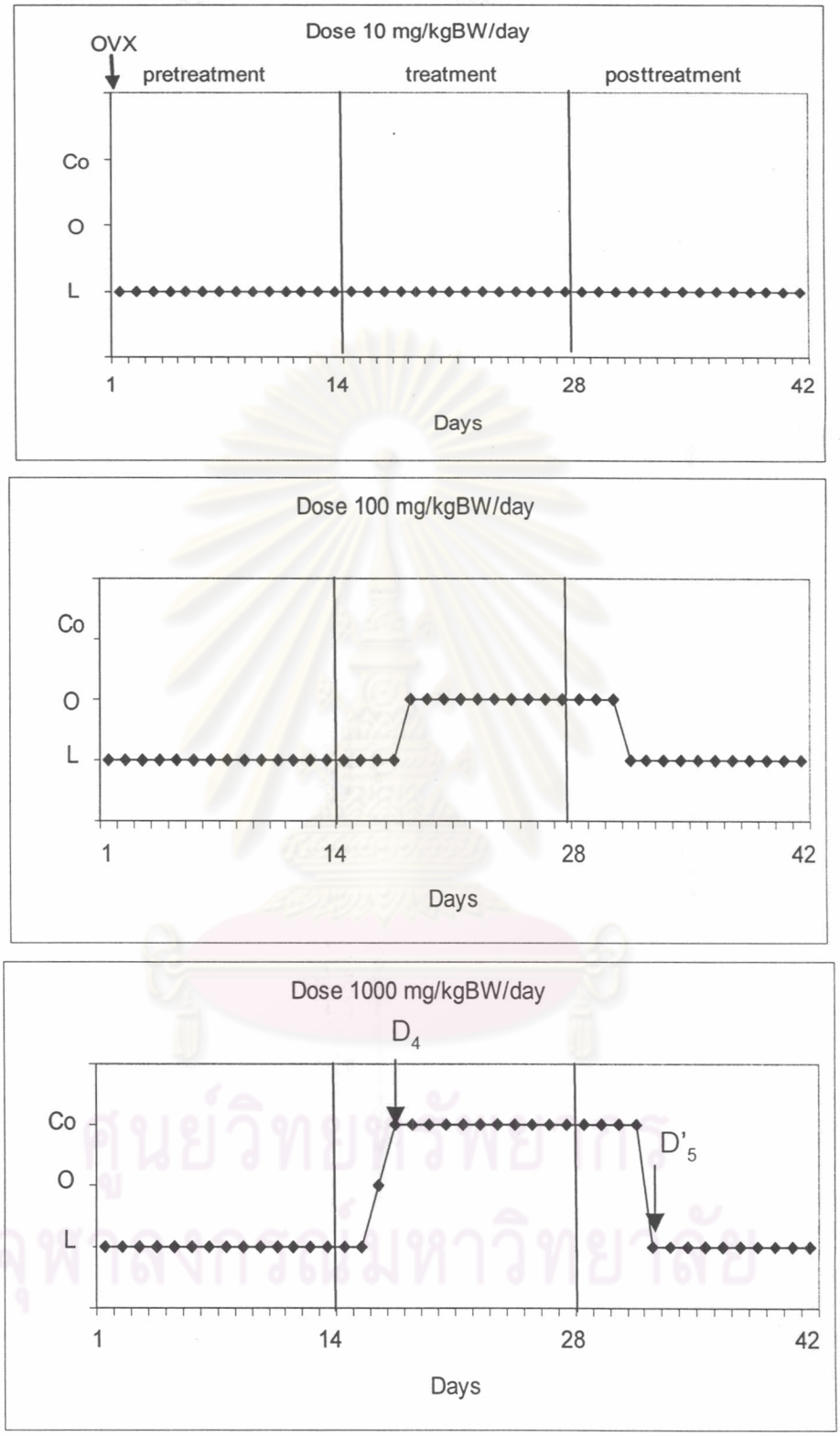

Figure 4-17. Changes of vaginal epithelium cells in rats treated with 10, 100 and 1,000 mg/kg BW/day of *P. mirifica* powder derived from the tuber collected from **Uthai thani** (Co= cornified cells, O= nucleated cells, L= leucocyte cells, D=Day with appearance of cornified cells, D'= Day with appearance of leucocyte cells).

The northeastern part of Thailand.

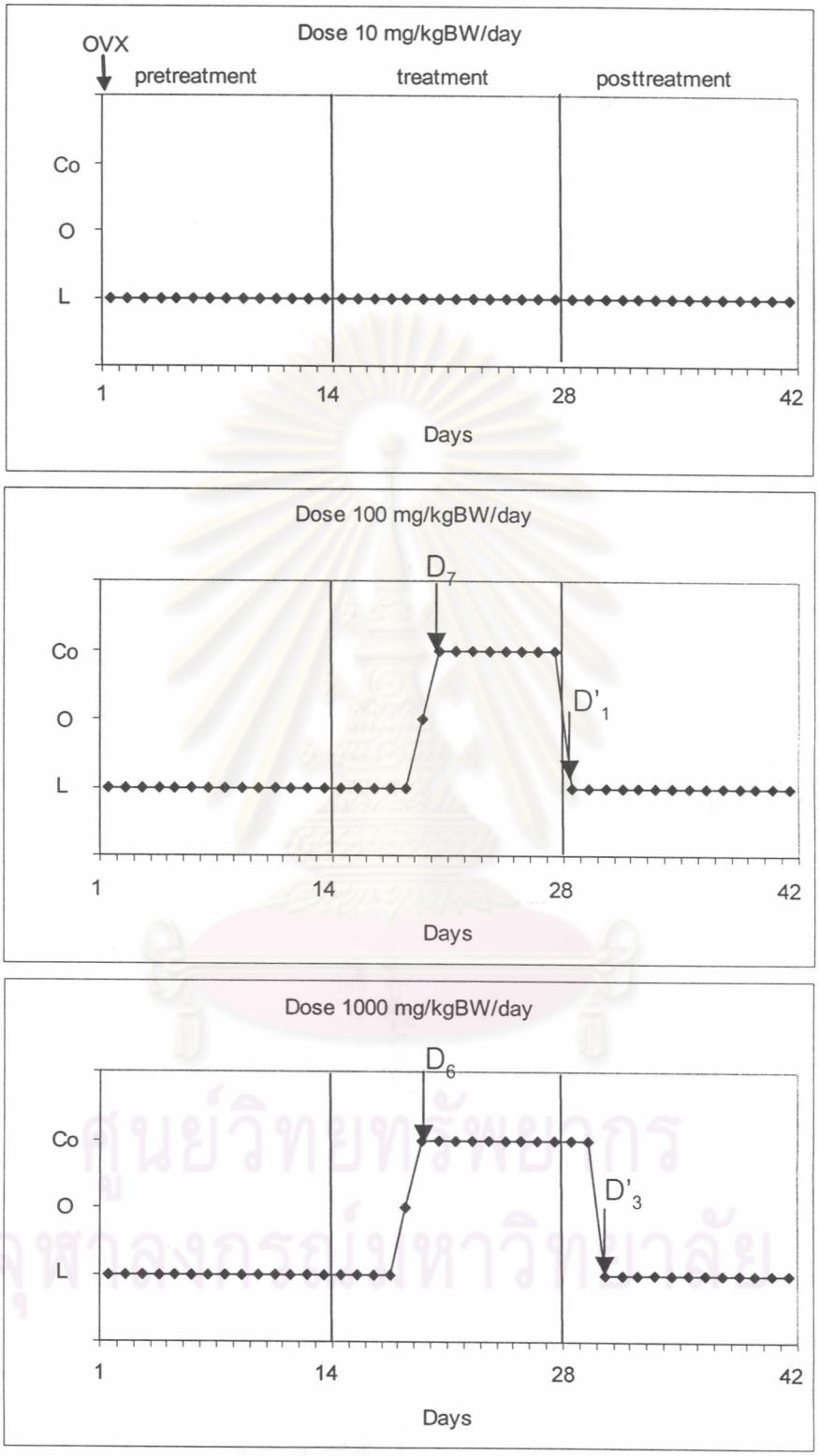

Figure 4-18. Changes of vaginal epithelium cells in rats treated with 10, 100 and 1,000 mg/kg BW/day of *P. mirifica* powder derived from the tuber collected from **Sakon Nakhon** (Co= cornified cells, O= nucleated cells, L= leucocyte cells, D=Day with appearance of cornified cells, D'= Day with appearance of leucocyte cells).

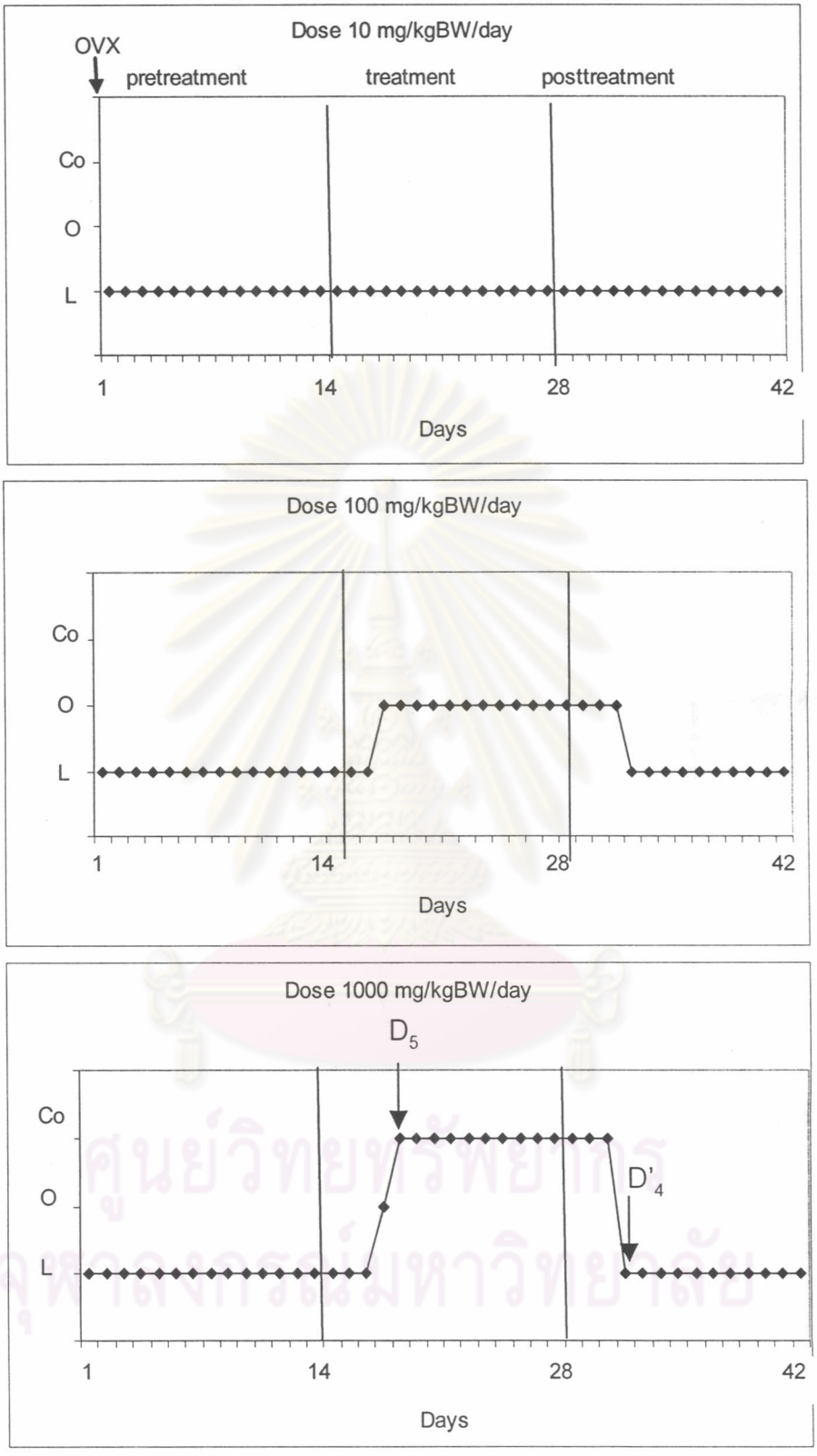

Figure 4-19. Changes of vaginal epithelium cells in rats treated with 10, 100 and 1,000 mg/kg BW/day of *P. mirifica* powder derived from the tuber collected from **Nong Bua Lam Phu** (Co= cornified cells, O= nucleated cells, L= leucocyte cells, D=Day with appearance of cornified cells, D'= Day with appearance of leucocyte cells).

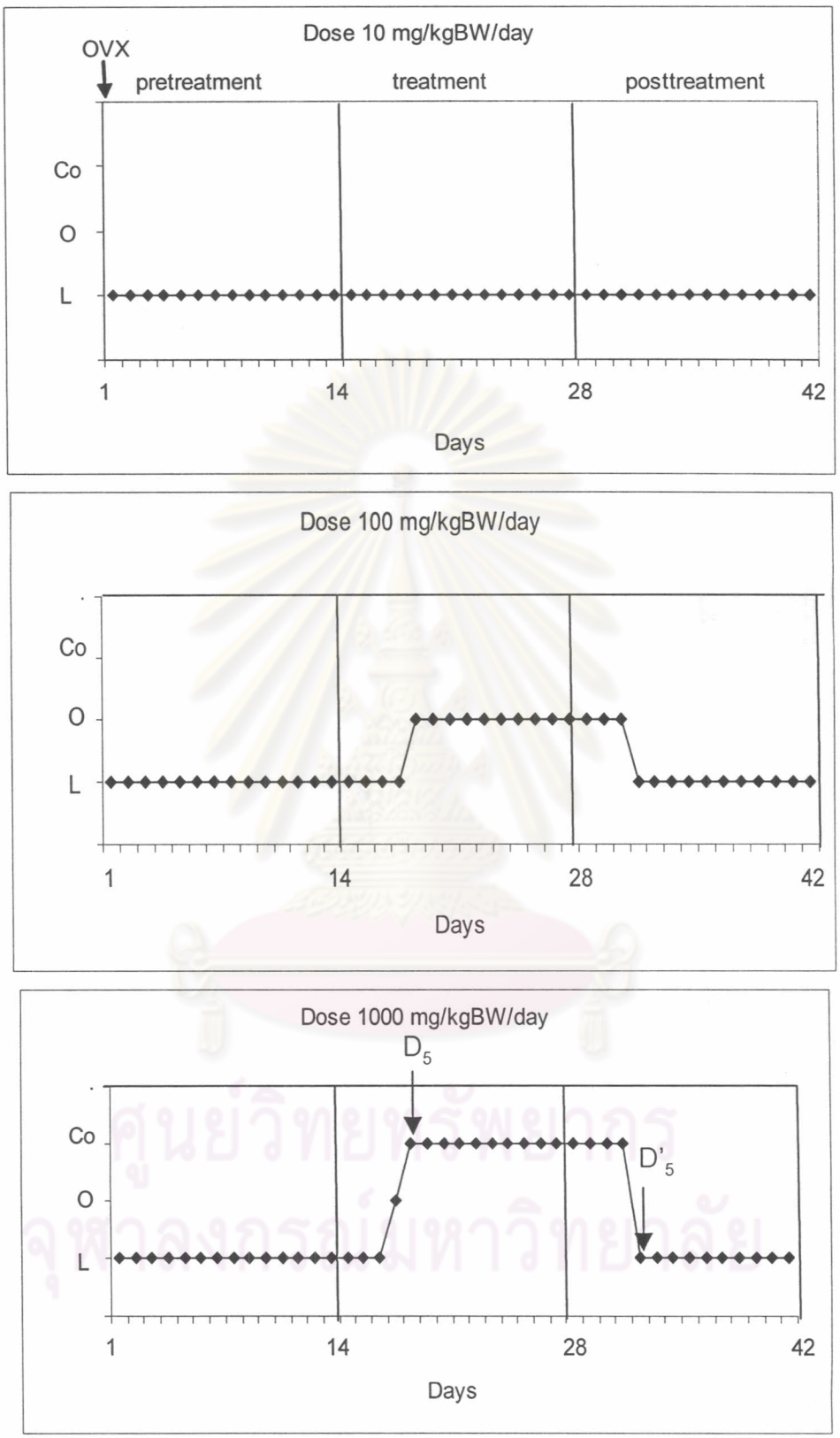

Figure 4-20. Changes of vaginal epithelium cells in rats treated with 10, 100 and 1,000 mg/kg BW/day of *P. mirifica* powder derived from the tuber collected from **Chaiyaphum** (Co= cornified cells, O= nucleated cells, L= leucocyte cells, D=Day with appearance of cornified cells, D'= Day with appearance of leucocyte cells)

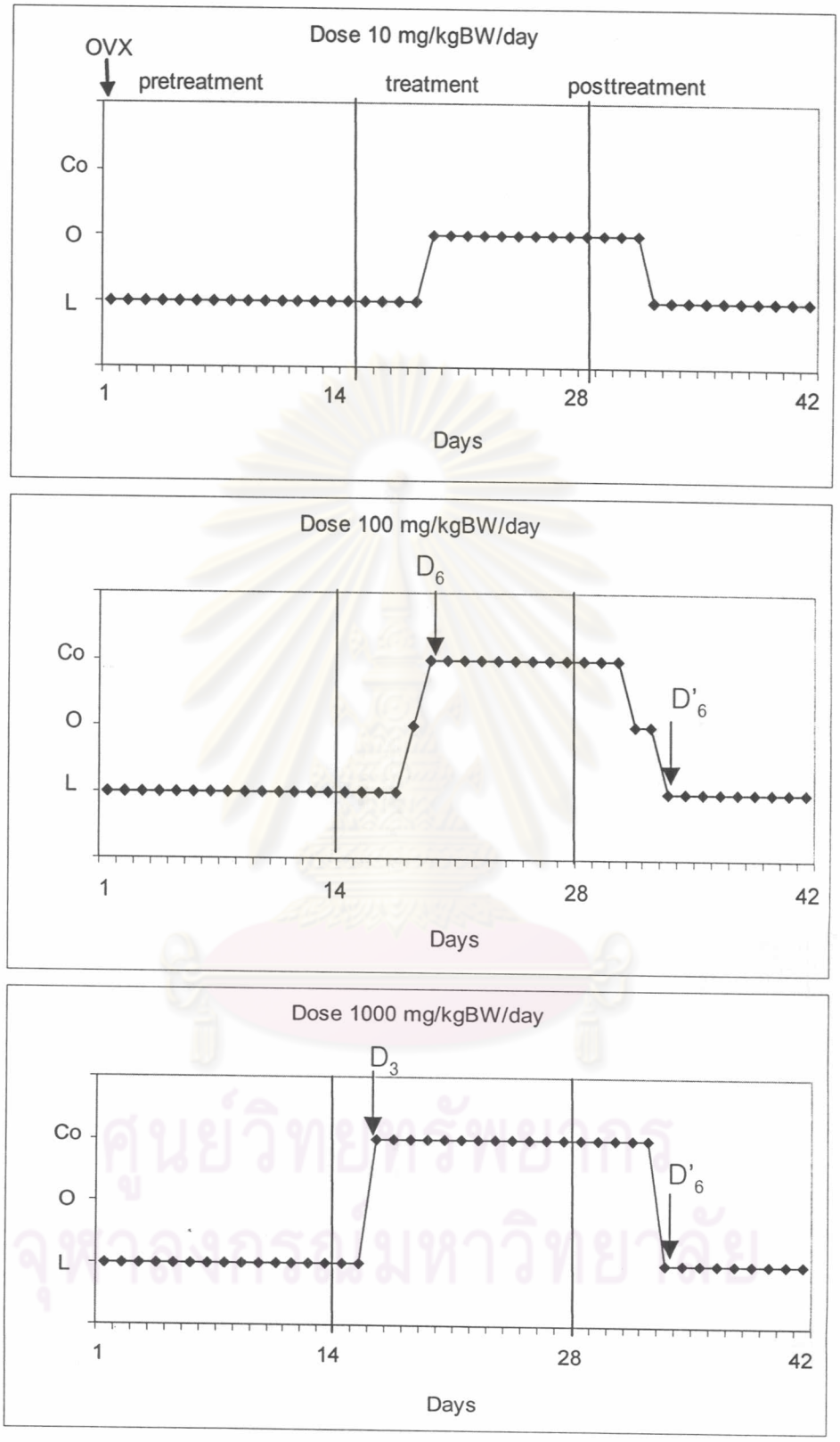

Figure 4-21. Changes of vaginal epithelium cells in rats treated with 10, 100 and 1,000 mg/kg BW/day of *P. mirifica* powder derived from the tuber collected from **Nakorn Ratchasima** (Co= cornified cells, O= nucleated cells, L= leucocyte cells, D=Day with appearance of cornified cells, D'= Day with appearance of leucocyte cells).

The central part of Thailand.

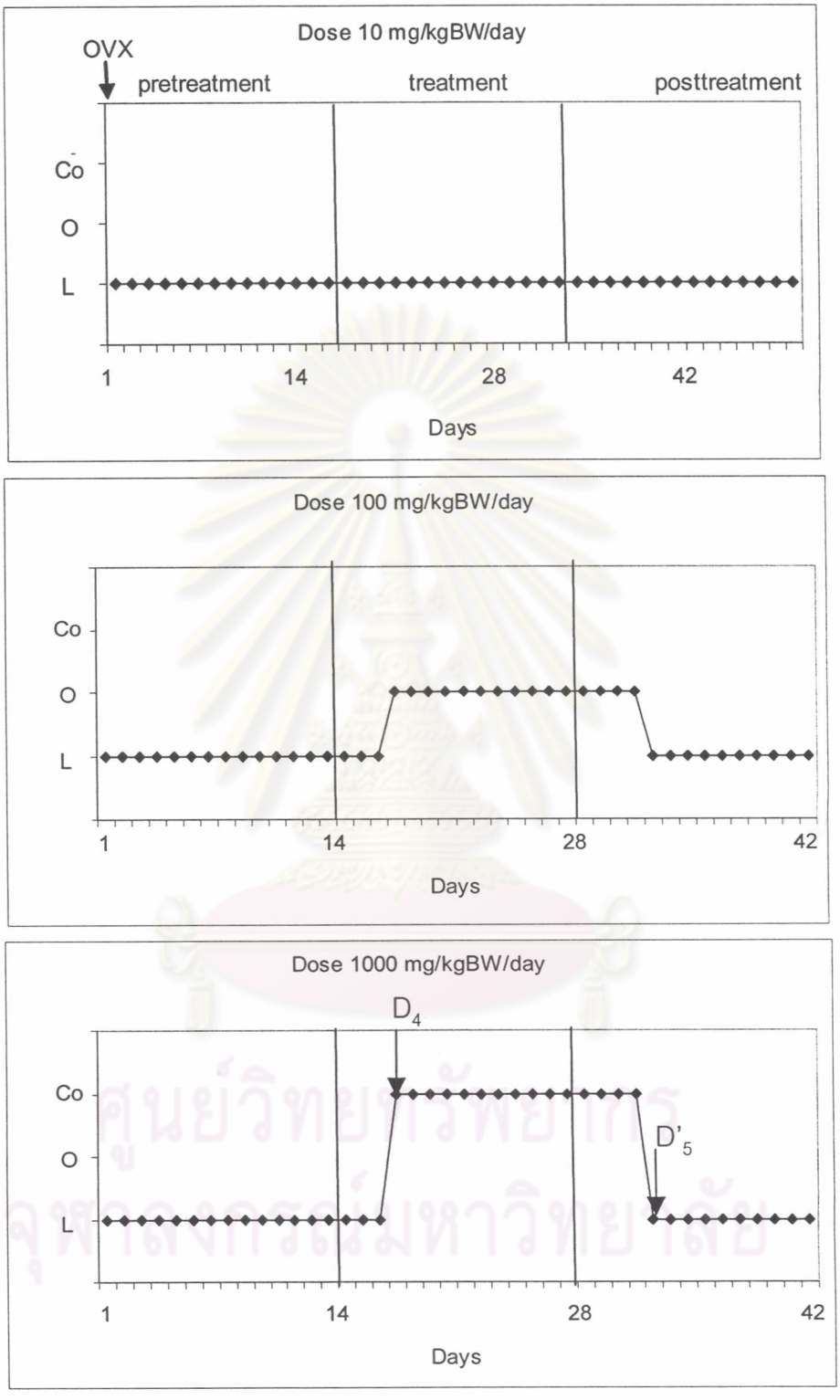

Figure 4-22. Changes of vaginal epithelium cells in rats treated with 10, 100 and 1,000 mg/kg BW/day of *P. mirifica* powder derived from the tuber collected from **Lopburi** (Co= cornified cells, O= nucleated cells, L= leucocyte cells, D=Day with appearance of cornified cells, D'= Day with appearance of leucocyte cells).

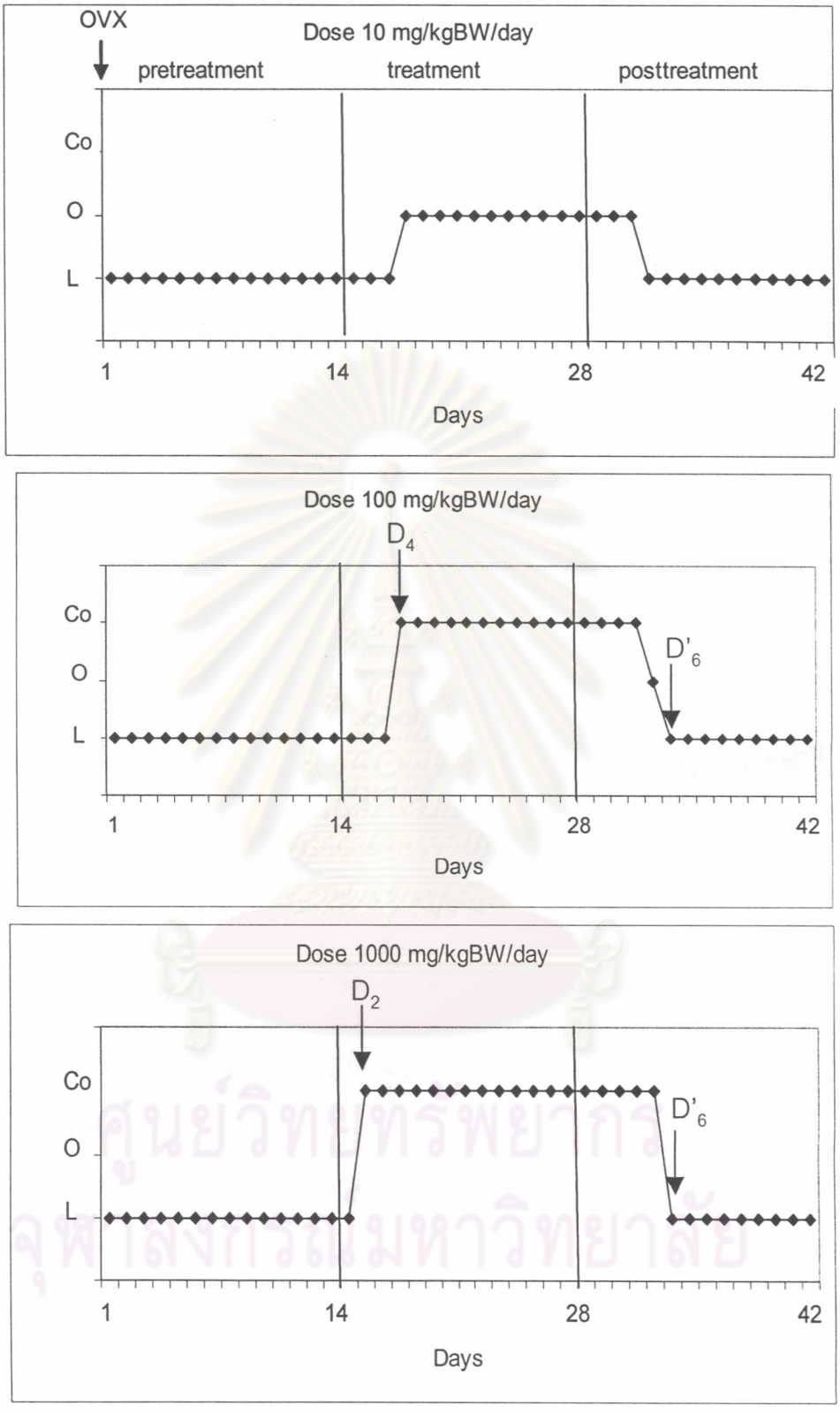

Figure 4-23. Changes of vaginal epithelium cells in rats treated with 10, 100 and 1,000 mg/kg BW/day of *P. mirifica* powder derived from the tuber collected from Kanchanaburi (Co= cornified cells, O= nucleated cells, L= leucocyte cells, D=Day with appearance of cornified cells, D'= Day with appearance of leucocyte cells).

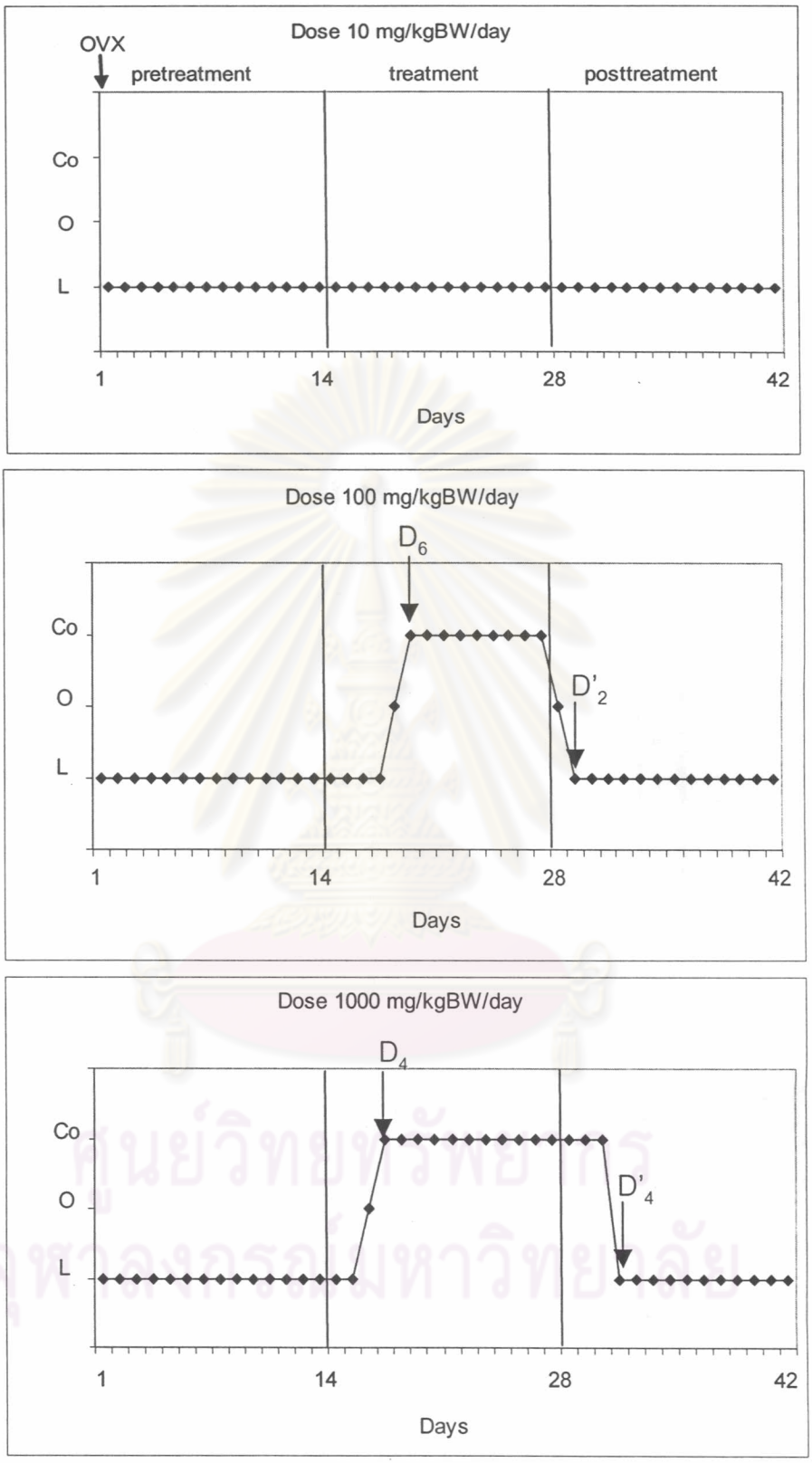

Figure 4-24. Changes of vaginal epithelium cells in rats treated with 10, 100 and 1,000 mg/kg BW/day of *P. mirifica* powder derived from the tuber collected from **Phrachin Buri** (Co= cornified cells, O= nucleated cells, L= leucocyte cells, D=Day with appearance of cornified cells, D'= Day with appearance of leucocyte cells).

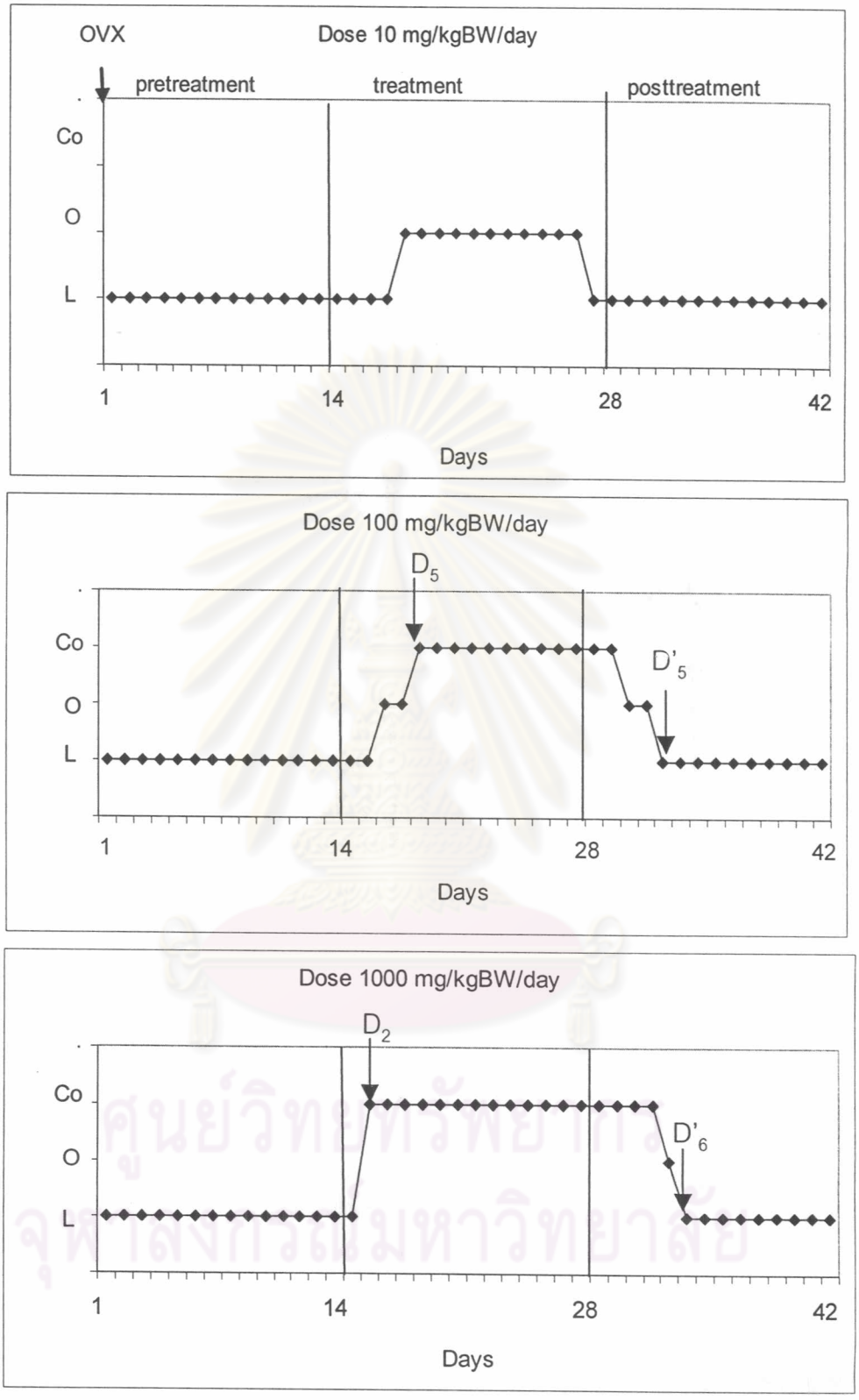

Figure 4-25. Changes of vaginal epithelium cells in rats treated with 10, 100 and 1,000 mg/kg BW/day of *P. mirifica* powder derived from the tuber collected from **Ratchaburi** (Co= cornified cells, O= nucleated cells, L= leucocyte cells, D=Day with appearance of cornified cells, D'= Day with appearance of leucocyte cells).

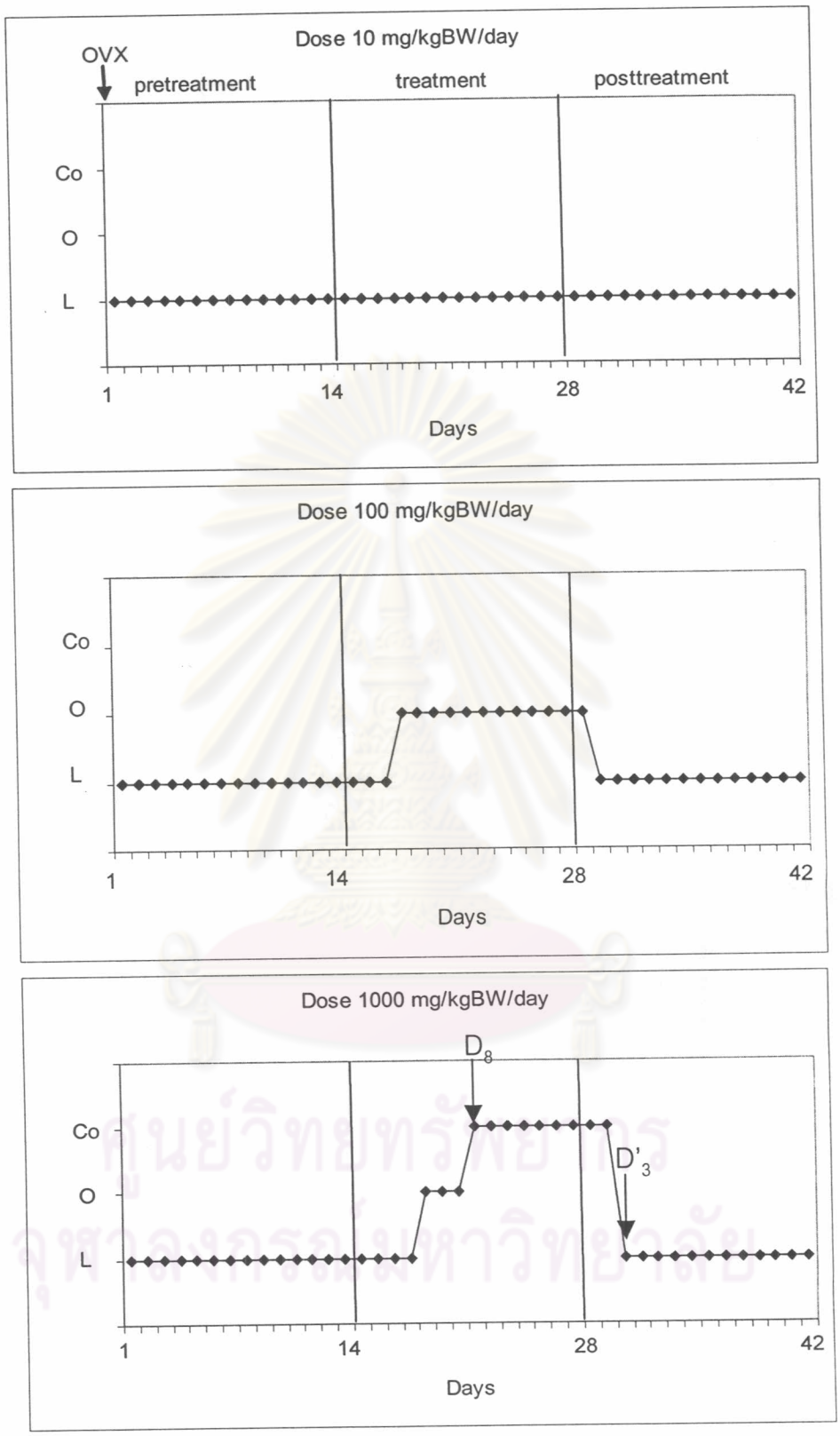

Figure 4-26. Changes of vaginal epithelium cells in rats treated with 10, 100 and 1,000 mg/kg BW/day of *P. mirifica* powder derived from the tuber collected from **Phetchaburi** (Co= cornified cells, O= nucleated cells, L= leucocyte cells, D=Day with appearance of cornified cells, D'= Day with appearance of leucocyte cells).

The southern part of Thailand.

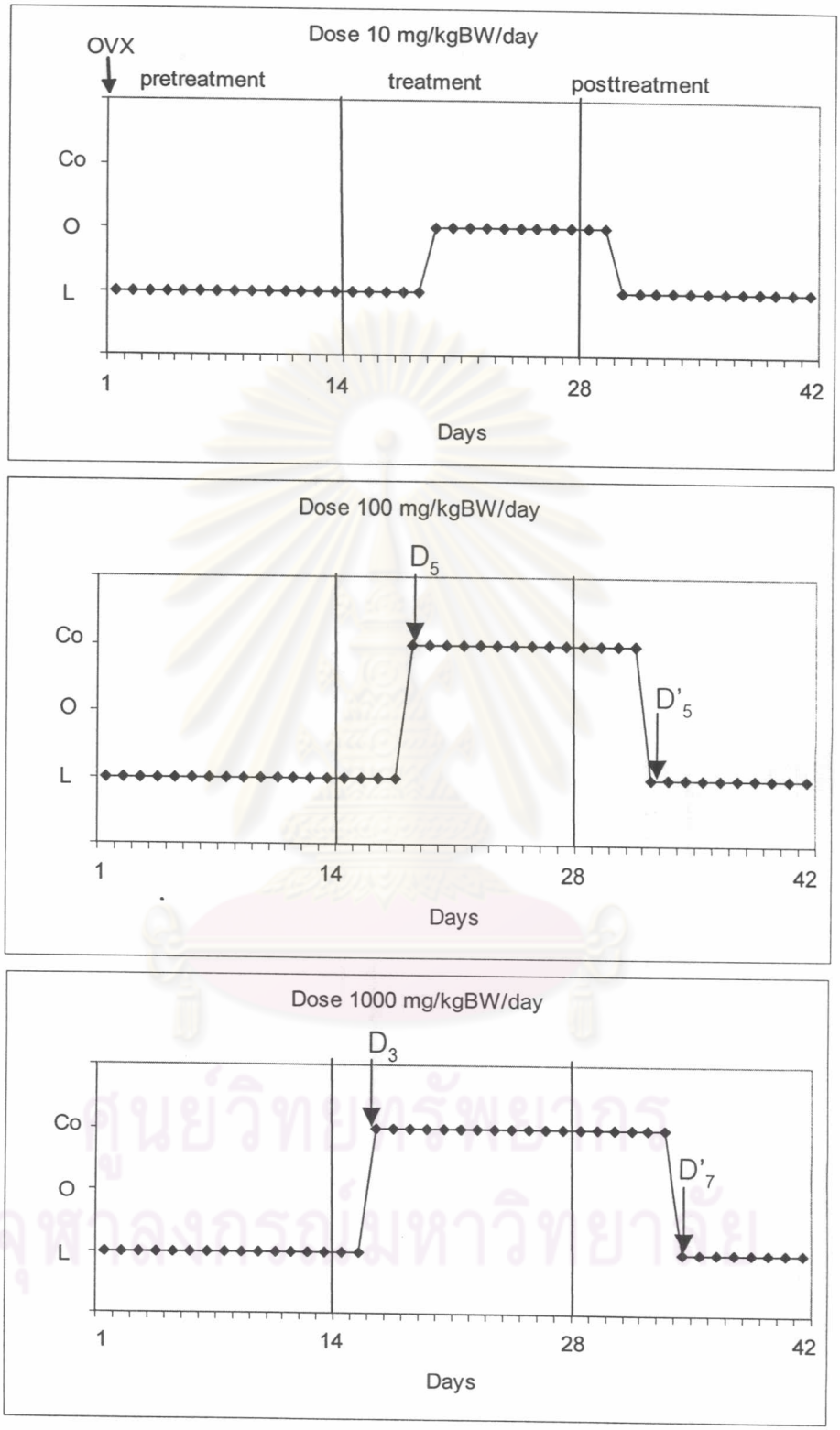

Figure 4-27. Changes of vaginal epithelium cells in rats treated with 10, 100 and 1,000 mg/kg BW/day of *P. mirifica* powder derived from the tuber collected from **Chumphon** (Co= cornified cells, O= nucleated cells, L= leucocyte cells, D=Day with appearance of cornified cells, D'= Day with appearance of leucocyte cells).

From those changes of vaginal epithelium cells in rats treated with 10, 100 and 1,000 mg/kg BW/day of *P. mirifica* powder, there are differences of the appearance of cornified cell during treatment period. It could be summerized in Table 4-1. The appearances of leucocyte cells during posttreatment were also summerized in Table 4-2.

Table 4-1. Day of appearance of cornified cells in rats after treated with *Pueraria mifica* collected from 25 provinces, 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).

		Day of appearance of cornified cells		
Region	Province	Dose 10 mg/kgBW/day	Dose 100 mg/kg BW/day	Dose 1,000 mg/kg BW/day
Northern	Chiang Rai	N^L	5	3
	Mae Hong Son	N^L	4	4
	Phayoa	N^L	N^L	5
	Nan	N^L	N^L	4
	Lumpang	N^L	N^L	5
	Phrae	N^L	N^L	4
	Lumphun	N^L	N^L	5
	Uttharadith	N^L	N^L	8
	Sukhothai	N^L	5	4
	Tak	N^L	N^L	4
	Phitsanulok	N^L	6	2
	Phetchabun	N^L	6	3
	Kampaeng Phet	N^L	6	4
	Nakorn Sawan	N^L	5	3
	Uthai thani	N^L	N^L	4
Mean ±SE (n=15)		-	9.33 ± 1.16^{ns}	4.13 ± 0.35^{ns}

North eastern	Sakon Nakhon	N^L	7	6
	Nong Bua Lam Phu	N^L	N^L	5
	Chaiyaphum	N^L	N^L	5
	Nakorn Ratchasima	N^L	6	3
Mean ± SE (n=4)		-	10.25 ± 2.17 ^{ns}	4.75±0.62 ^{ns}
Central	Lop Buri	N^L	N^L	4
	Kanchanaburi	N^L	4	2
	Phrachin Buri	N^L	6	4
	Ratchaburi	N^L	5	2
	Phetchaburi	N^L	N^L	8
Mean ± SE (n=5)		-	8.60 ± 2.22 ^{ns}	4.00±1.09 ^{ns}
Southern	Chumphon	N^L	5	3
Control	Distilled water (0.7ml/day)	N^L		
	17β - estradiol (200 μg /100gBW)	2		

ns= non significant difference among northern, northeastern and central parts

From the statistical analysis of the length (day) of appearance of cornified cells among 3 regions; northern, northeastern and central Thailand in Table 4-1, it was found that *P. mirifica* from 3 regions did not show any effect of vaginal epithelium in the dosage of 10 mg/kgBW/day. There were no statistical differences among those 3 regions in dosage of 100 and 1,000 mg/kgBW/day. The dosage 100 mg/kgBW/day from the central part of Thailand, however, tended to the higher estrogenic activity (earlier appearance of cornified cells) than the north and northeastern parts, respectively. The stimulation of vaginal cornification by *P. mirifica* in the dosage of 1,000 mg/kgBW/day also showed the tend of higher estrogenic activity in central > north> northeast, similar to the dosage of 100 mg/kgBW/day. The results of southern part from Chumphon province was not used for statistical analysis, because of only 1 province, it, however, showed a high estrogenic activity of the appearance of cornified cells in both of 100 and 1,000 mg/kgBW/day.

Table 4-2. Day of appearance of leucocyte cells in rats after treated with of *Pueraria mifica* collected from 25 provinces, distilled water and 17β- estradiol during posttreatment period (N^L = No cornified cells, and leucocyte cells were found throughout the experiment period and 1 days were used as a number for statistical analysis).

		Day of appearance of leucocyte cells		
Regions	Province	Dose10 mg/kgBW/day	Dose 100 mg/kgBW/day	Dose 1,000 mg/kgBW/day
Northern	Chiang Rai	N^L	5	6
	Mae Hong Son	N^L	3	5
	Phayoa	N^L	N^L	4
	Nan	N^L	N^L	5
	Lumpang	N^L	N^L	5
	Phrae	N^L	N^L	5
	Lumphun	N^L	N^L	5
	Uttharadith	N^L	N^L	3
	Sukhothai	N^L	4	6
	Tak	N^L	N^L	7
	Phitsanulok	N^L	4	6
	Phetchabun	N^L	2	4
	Kampaeng Phet	N^L	4	6
	Nakorn Sawan	N^L	2	6
	Uthai thani	N^L	N^L	5
Mean ± SE (n=15)		-	2.13 ± 0.38^{ns}	5.2 ± 0.26^{ns}
north eastern	Sakon Nakhon	N^L	1	3
	Nong Bua Lam Phu	N^L	N^L	4
	Chaiyaphum	N^L	N^L	5
	Nakorn Ratchasima	N^L	6	6
Mean ± SE (n=4)		-	2.25 ± 2.16^{ns}	4.50 ± 0.64^{ns}

Central	Lop Buri	N^L	N^L	5
	Kanchanaburi	N^L	6	6
	Phrachin Buri	N^L	2	4
	Ratchaburi	N^L	5	6
	Phetchaburi	N^L	N^L	3
Mean± SE(n=5)		-	3.00± 1.04 ^{ns}	4.80±0.58 ^{ns}
Southern	Chumphon	N^L	5	7
Control	Distilled water (0.7ml/day)	N^L		
	17β - estradiol (200 μg /100gBW)	7		

ns= non significant difference among northern, northeastern and central parts

From the statistical analysis of the day of appearance of leucocytes cells among 3 regions; northern, northeastern and central Thailand in Table 4-2, it was found that *P. mirifica* collected from those 3 regions did not show any estrogenic activity in dosage of 10 mg/kgBW/day. In doses of 100 and 1,000 mg/kgBW/day of *P. mirifica* were no statistical differences among those 3 regions. However, *P. mirifica* collected from the central Thailand tended to show a longer estrogenic activity (longer day of leucocyte apperrances) than the northeastern and northern parts, respectively. On the others hand, 1,000 mg/kgBW/day of *P. mirifica* tended to show a longer estrogenic activity in the northern part than the central and the northeastern parts, respectively

When the data of Table 4-1 and 4-2 were combined and calculated for the duration of the appearance of cornified cells during the treatment and posttreatment periods, the estrogenic activity of *P. mirifica* collected from 25 provinces could be ranked according to the regions as shown in Table 4-3. Although there were no statistical differences among those 3 regions in *P. mirifica* doses of 100 and 1,000 mg/kgBW/day, the *P. mirifica* collected from the central part tended to show the highest estrogenic activity (the longest duration of appearances of cornified cells) in both of doses of 100 and 1,000 mg/kgBW/day. Comparison among 5 provinces in the central part, *P. mirifica* collected from Kanchanaburi province showed the highest estrogenic

activity in both doses. When the estrogenic activity was ranked according to the appearances of cornified cells, *P. mirifica* collected from the northern part tended to have a higher estrogenic activity than that of the northern part, and Phetchaburi showed the lowest activity in both doses. Comparison among 15 provinces in the northern part, *P. mirifica* collected from Mae Hong Son and Sukhothai had the highest estrogenic activity in the dose of 100 mg/kgBW/day. On contrary, *P. mirifica* collected from Chiang Rai and Phitsanulok provinces had the highest estrogenic activity in the dose of 1,000 mg/kgBW/day. Uttharadith showed the lowest estrogenic activity in both doses.

Table 4-3 The duration of appearance of cornified cells during treatment and posttreatment periods.

Region	Provinces	Total day of cornified cells (dose 10 mg/kgBW/day)	Total day of cornified cells (dose 100 mg/kgBW/day)	Total day of cornified cells (dose 1,000 mg/kgBW/day)
Northern	Chaing Rai	0	12	17
	Mae Hong Son	0	13	15
	Phayoa	0	0	12
	Nan	0	0	14
	Lampang	0	0	14
	Phrae	0	0	14
	Lumphun	0	0	13
	Uttharadith	0	0	8
	Sukhothai	0	13	15
	Tak	0	0	16
	Phitsanulok	0	10	17
	Phetchabun	0	9	14
	Kampaeng Phet	0	12	16
	Nakorn Sawan	0	10	15
	Uthai thani	0	0	15
Mean± SE (n=15)		0	5.26 ± 1.52 ^{ns}	14.33±0.57 ^{ns}
North eastern	Sakon Nakhon	0	8	11
	Nong Bua Lam - Phu	0	0	13
	Chaiyaphum	0	0	14
	Nakorn - Ratchasima	0	12	17
Mean± SE (n=4)		0	5.00±3.00 ^{ns}	13.75± 1.25 ^{ns}

Central	Lop Buri	0	0	15
	Kanchanaburi	0	15	18
	Phrachin Buri	0	9	14
	Ratchaburi	0	13	17
	Phetchaburi	0	0	9
Mean± SE (n=5)			7.40±3.17 ^{ns}	14.60 ± 1.56 ^{ns}
South	Chumphon	0	14	18
Control group	Distilled water (0.7ml/day)	0		
	17β - estradiol (200 μg 100gBW)	19		

ns= non significant difference among northern, northeastern and central parts

To compare the estrogenic activity of *P. mirifica* among three dosages, the length of the apperance of cornified cells after *P. mirifica* treatment and posttreatment were compared. The dosage of 10 mg/kgBW/day did not show a significant difference from the negative control. In the dose 100 mg/kgBW/day, it was significant longer than the negative control and significantly than from the positive control. The dosage 1,000 mg/kgBW/day exhibited the highest estrogenic activity but it still was significantly lower than the positive control ($P<0.05$) (Figure 4- 28).

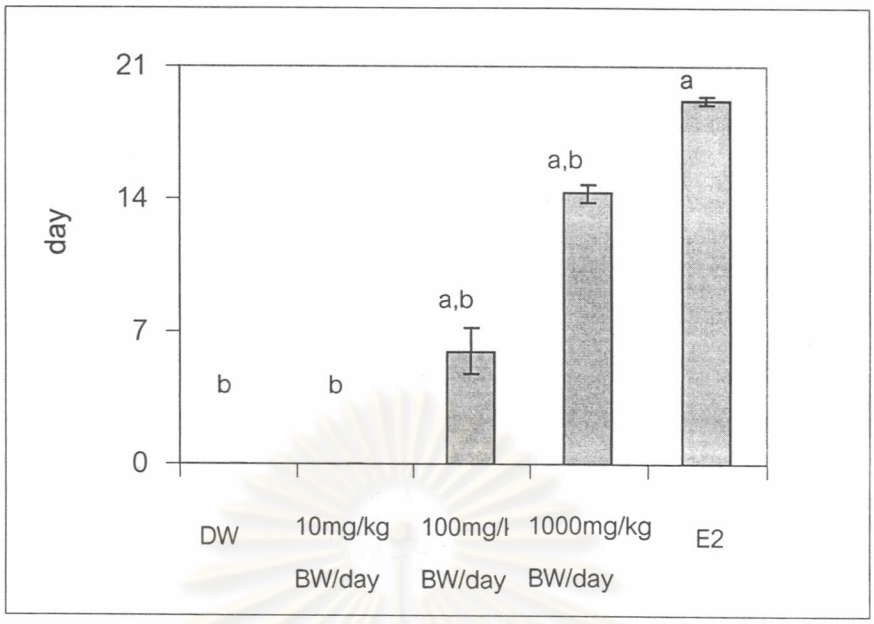

Figure 4-28. The mean ± SE of the length of the appearance of cornified cells during the 28-day period of *P. mirifica* treatment and posttreatment.

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

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

The estrogenic activity of *P. mirifica* in Thailand in the dose of 10, 100, 1,000 mg/kgBW/day were mapped, related to the locations of collection, and shown in Figure 4-29, 4-30, 4-31.

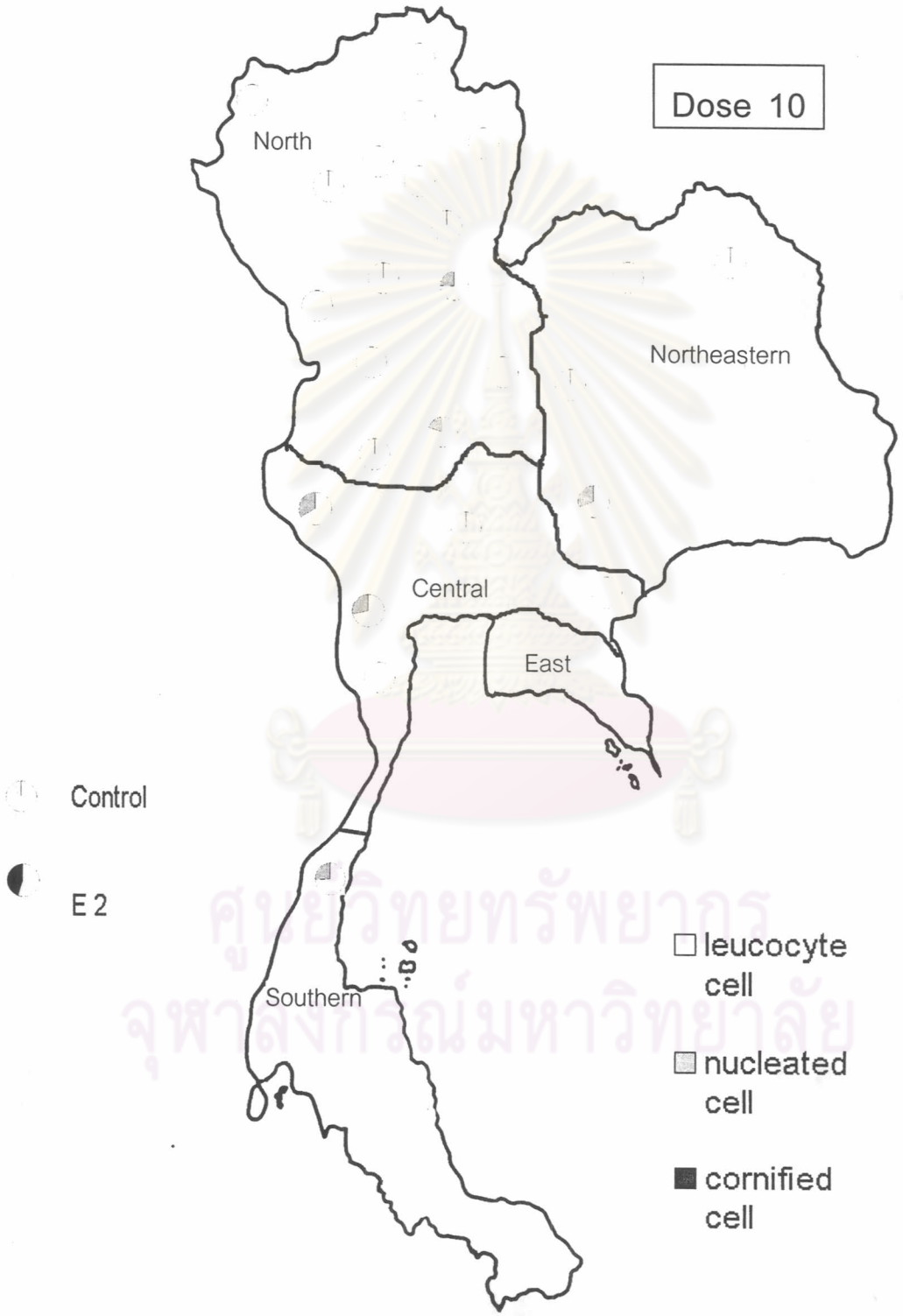

Figure 4-29. Map of changes of vaginal epithelium cells in rats after treated with 10 mg/kg BW day of *P. mirifica* related to the locations of collection.

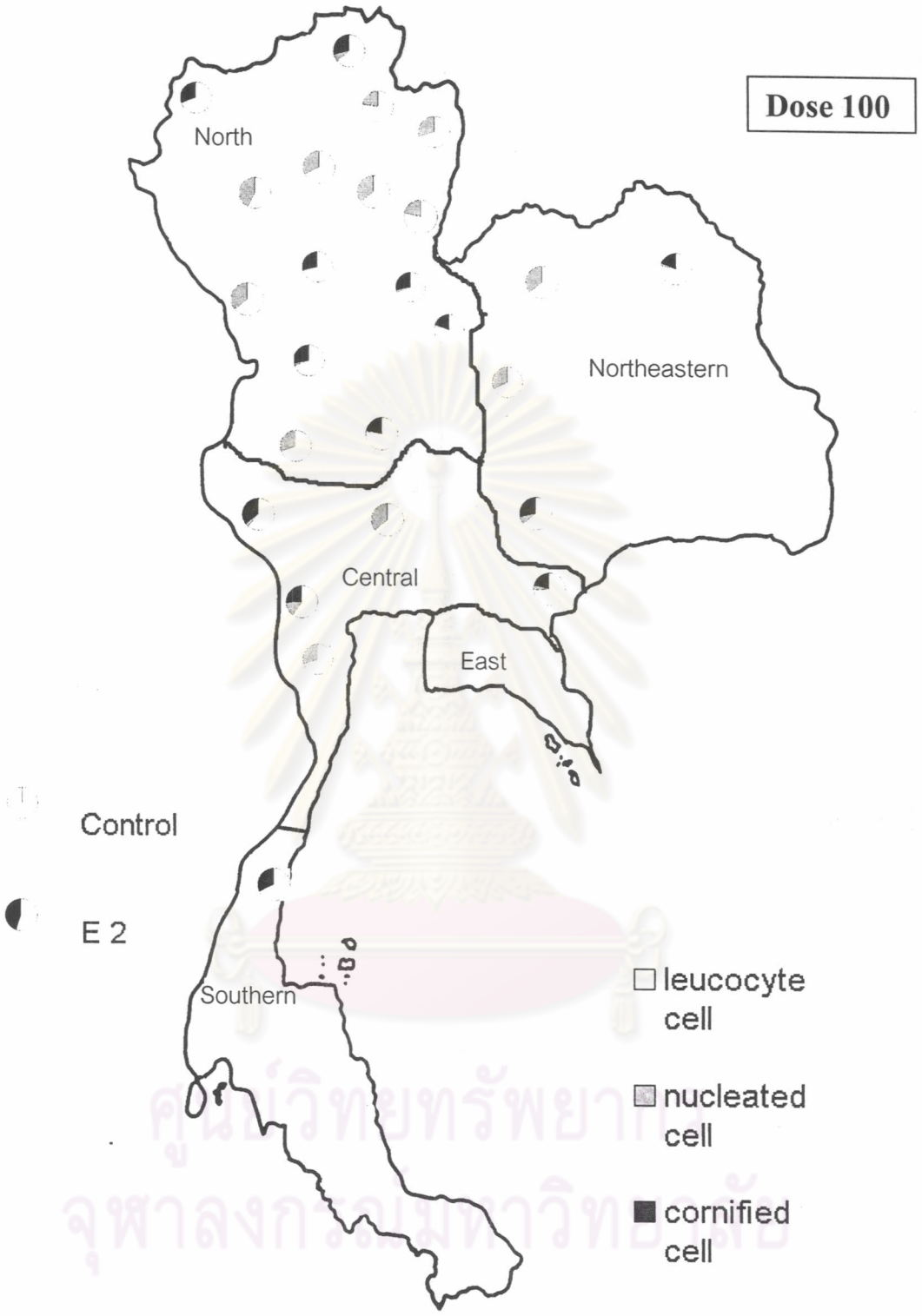

Figure 4-30. Map of changes of vaginal epithelium cells in rats after treated with 100 mg/kg BW day of *P. mirifica* related to the locations of collection.

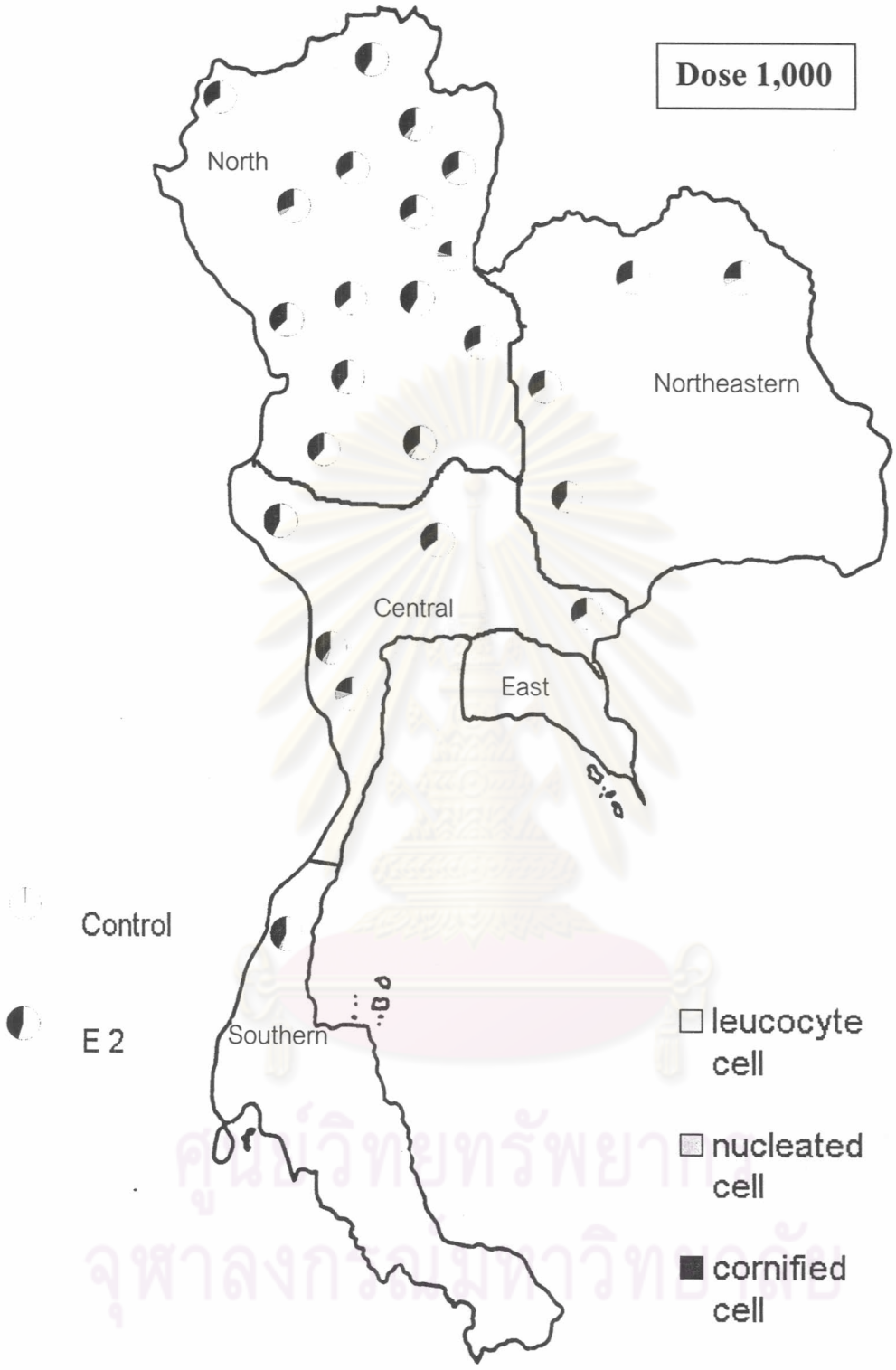

Figure 4-31. Map of changes of vaginal epithelium cells in rats after treated with 1,000 mg/kg BW day of *P. mirifica* related to the locations of collection.

2. Effect of *P. mirifica* on the percentage of cornified cells in ovariectomized rats

From the result of vaginal cornification in rats after treated with 100 mg/kgBW/day of *P. mirifica*, it was found that the earliest response is on D_4 after treated with *P. mirifica* collected from Kanchanaburi (central) and Mae Hong Son (northern). The latest response of vaginal cornification was found on D_8 in rats after treated with 1,000 mg/kgBw/day of *P. mirifica* collected from Phetchaburi (central) and Uttharadith (northern). These results agreed with the day of appearance of leucocytes cells during posttreatment period and the duration of appearance of cornified cells during both of treatment and posttreatment periods.

To rank the highest and lowest estrogenic activity in those *P. mirifica* cultivars, the vaginal smear cells were counted and calculated for the percentage of cornified cells. One-hundred vaginal smear cell were randomly counted for all of Co, O and L cell types. The percentage of cornified cells was calculated. Figure 4-32 and 4-33 presented mean $\pm$ SE of the percentage of cornified cell from 5 rats treated with *P. mirifica* compared with the positive and negative control groups. To find out the highest estrogenic activity, the percentage of cornified cells counted on D_4 after 100 mg/kgBw/day of *P. mirifica* treatment was compared between Kanchanaburi and Mae Hong Son provinces. It was found that the percentage of cornified cells in rats after feeding with *P. mirifica* collected from Kanchanaburi (81.20 ± 3.12) is higher than Mae Hong Son (52.80 ± 4.09). That is, the estrogenic activity of *P. mirifica* collected from Kanchanaburi is the highest among those 25 provinces. However, the percentage of cornified cells counted on D_4 after feeding with 100 mg/kgBw/day of *P. mirifica* collected from Kanchanaburi was still lower than that of positive control group treated with 17β - estradiol, that is, the one hundred percent of cornified cells was found. In addition, the percentage of cornified cells count on D_8 after 1,000 mg/kgBw/day of *P. mirifica* treatment was compared between Phetchaburi and Uttharadith provinces, for the lowest estrogenic activity. It was found that the percentage of cornified cells in rats after feeding with *P. mirifica* collected from Uttharadith (79.32 ± 10.20) is lower than Phetchaburi (87.23 ± 3.10). That is the estrogenic activity of *P. mirifica* collected from Uttharadith is the lowest among those 25 provinces.

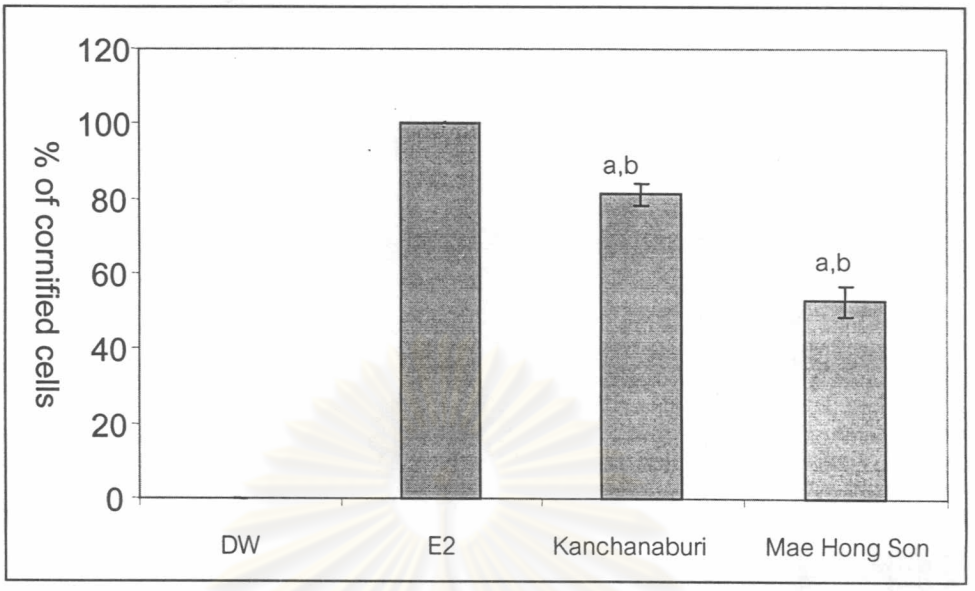

Figure 4-32 The percentage of cornified cell in rats counted on D_4 after feeding of *P. mirifica* ,distilled water and 17β - estradiol.

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

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

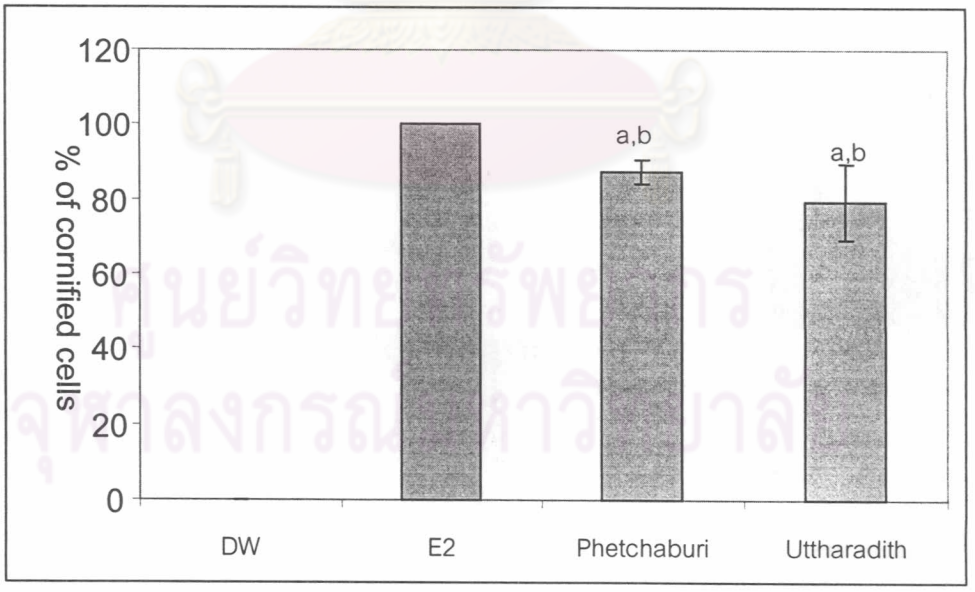

Figure 4-33 The percentage of cornified cell in rats counted on D_8 after feeding of *P. mirifica* , distilled water and 17β - estradiol.

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

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

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

The body weight of rats 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 is as follows;

1.Control group;

1) Negative control : The body weight of rats in the negative control group was significantly increased from day 1 presented in Figure 4-34

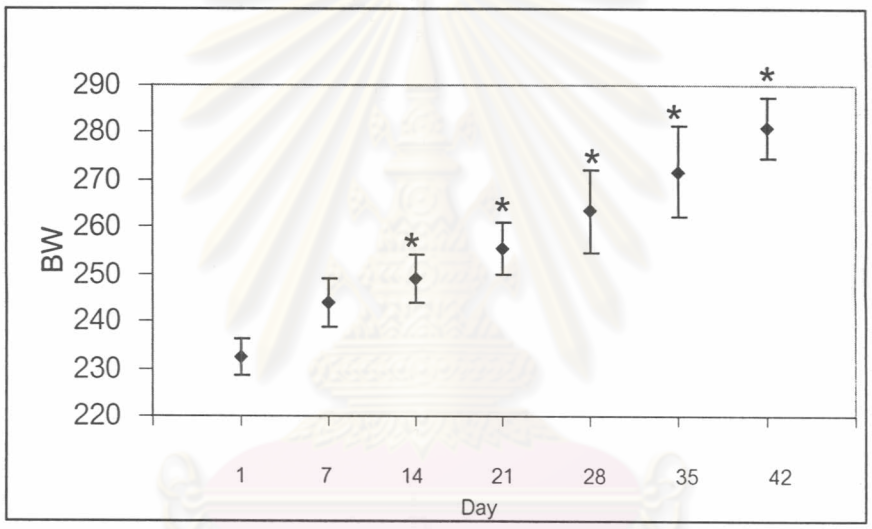

Figure 4-34 The body weight of rat treated with distilled water (* =significant difference from day 1).

2) 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 4-35

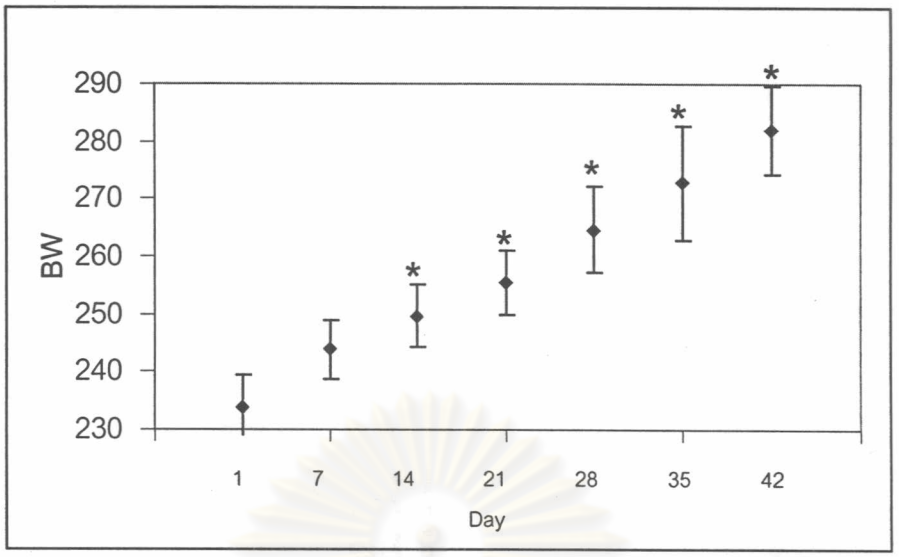

Figure 4-35. The body weight of rat treated with the 17β - estradiol (* =significant difference from day 1).

2. *P. mirifica* treatment group;

1) Dose 10 mg/kg BW/day (Figure 4-36)

In comparison with the negative control group, the body weight of rats were significant decrease from control group in day 35, 42 ($P<0.05$).

2) Dose 100 mg/kg BW/day (Figure 4-37)

The body weight were significant decrease from the control group in the day 21, 28, 35, 42 ($P<0.05$).

3) Dose 1,000 mg/kg Bw/day (Figure 4-38)

The body weight were significant decrease from the control group in the day 14, 21, 28, 35, 42 ($P<0.05$).

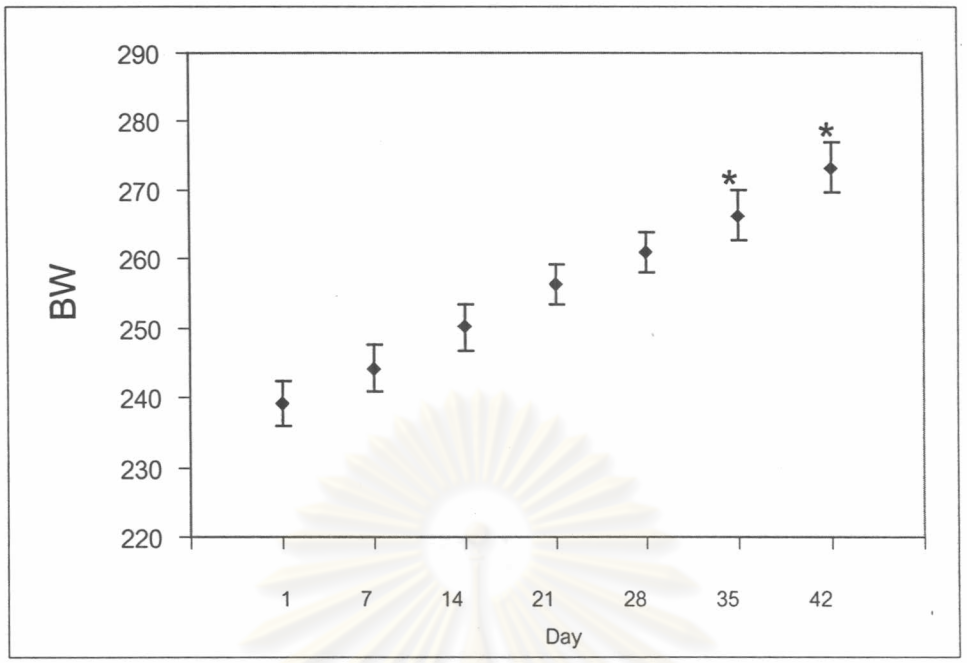

Figure 4-36. The body weight of rat treated with 10 mg/kg BW/day of *P. mirifica* on day 1, 7, 14, 21, 28 ,35, 42 (* =significant difference from negative control).

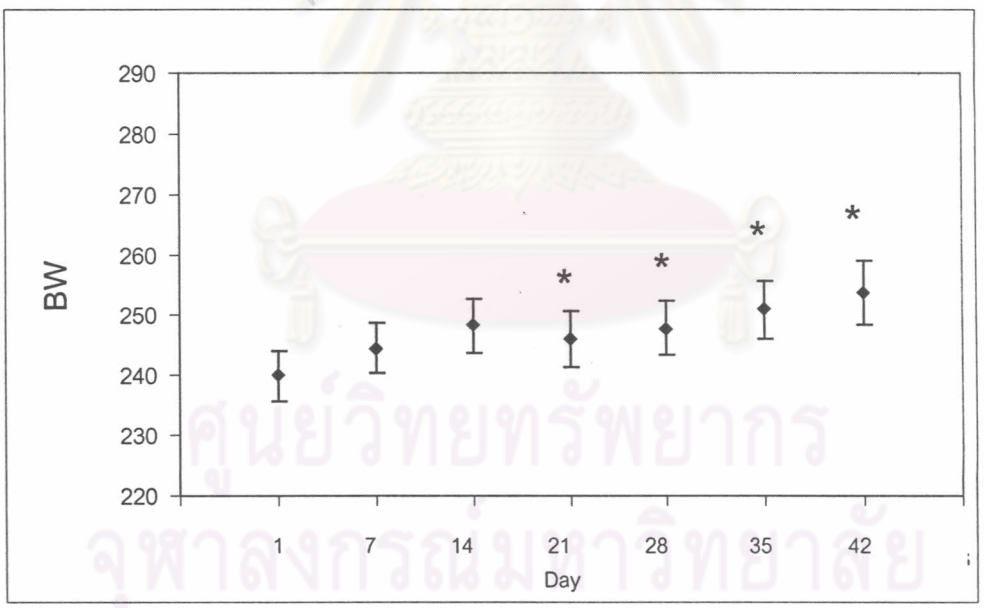

Figure 4-37. The body weight of rat treated with 100 mg/kg BW/day of *P. mirifica* on day 1, 7, 14, 21, 28 ,35, 42 (* =significant difference from negative control).

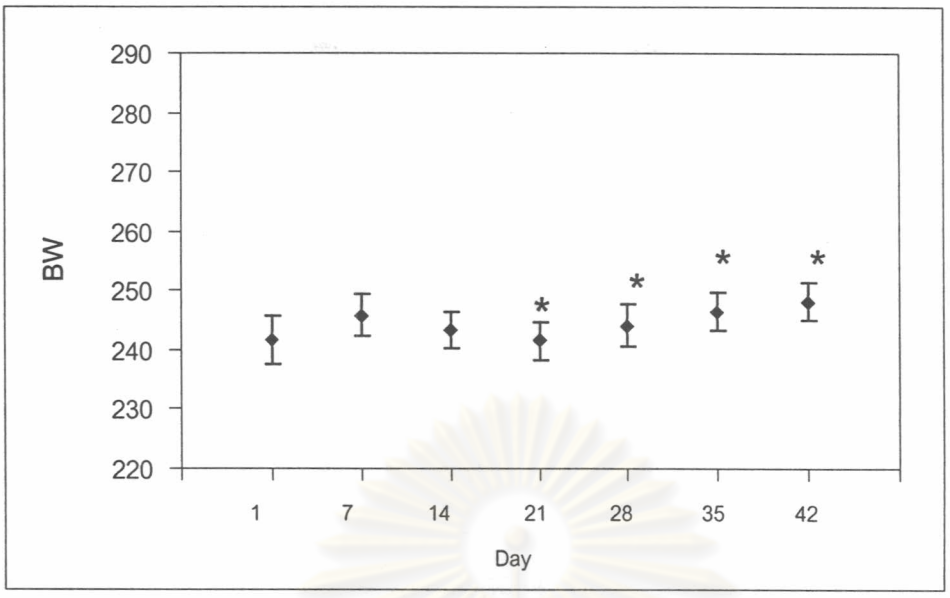

Figure 4-38. The body weight of rat treated with 1,000 mg/kg BW/day of *P. mirifica* on day 1, 7, 14, 21, 28 ,35, 42 (* = significant difference from negative control).

4. Effect of *P. mirifica* on uterine weight of ovariectomized rats

The results of uterine weight in rats treated with *P. mirifica*, 17β - estradiol and distilled water are presented in Table 4-4. The increment of uterine weight at the end of the post- treatment period (day 42) was agreed with changes of vaginal epithelium cell. The uterine weights of rats treated with *P. mirifica* collected from the central part is the highest. The uterine weights in rats treated with 10 mg/kgBW/day of *P. mirifica* collected from central was significantly higher the that of the northern and the northeastern parts. The uterine weight in rats treated with 100 mg/kg BW/day of *P. mirifica* were as follows; central> north ≥northeast. However, there were no significant differences of uterine weights in rats treated with 1,000 mg/kg BW/day of *P. mirifica* collected from those 3 regions. When the data of uterine weights of rats treated with *P. mirifica* collected from 25 provinces were pooled and compared between doses, the uterine weight was increased in a dose dependent manner as presented in Figure 4-39. The uterine weight of rats treated with 10 and 100 mg/kg BW/day of *P. mirifica* did not significantly differ from that of the negative control group (DW).

Table 4-4. Mean ± SE of the uterus weight after treated with *P. mirifica* collected from 25 provinces, distilled water and 17β - estradiol.

Regions	Provinces	Dose 10 mg/kgBW/day(mg)	Dose 100 mg/kgBW/day(mg)	Dose 1000 mg/kgBW/day(mg)
Northern	Chaing Rai	156.00±19.19	189.20±19.93	123.60±32.51
	Mae Hong Son	94.50±13.40	130.80±3.84	169.00±22.44
	Phayoa	128.40±3.98	147.40±10.22	144.90±8.49
	Nan	109.00±10.6	142.40±1.63	149.00±17.24
	Lampang	122.60±7.52	141.90±4.15	175.60±1.89
	Phrae	136.60±18.39	165.00±22.04	199.00±20.09
	Lumphun	121.40±11.52	131.60±14.38	160.20±1.38
	Uttharadith	144.80±21.93	164.60±17.79	177.40±8.15
	Sukhothai	97.60±8.61	162.80±17.65	134.80±3.81
	Tak	141.80±16.10	173.60±6.06	172.60±6.66
	Phitsanulok	112.00±6.83	153.60±12.47	182.00±35.12
	Phetchabun	124.40±12.81	124.00±15.10	169.40±5.22
	Kampaeng Phet	100.80±4.62	129.20±13.18	186.80±9.71
	Nakorn Sawan	120.80±8.77	144.00±1.12	170.80±30.73
	Uthai thani	115.40±6.05	165.80±9.39	203.30±19.33
Mean±SE		121.70±4.59 ^{ns}	151.10±4.84*	168.10±5.80*
North-eastern	Sakon Nakhon	123.00±16.24	135.80±16.37	176.20±13.53
	Nong Bua Lam - Phu	115.00±26.27	142.20±6.84	187.80±8.19
	Chaiyaphum	148.80±13.50	143.00±15.45	171.80±23.68
	Nakorn - Ratchasima	144.20±8.69	160.40±10.05	173.40±14.27

Mean±SE		132.70±8.16 ns	145.40±5.26 ns	177.30±3.61*
Central	Lop Buri	137.40±11.72	201.40±11.07	202.00±10.66
	Kanchanaburi	165.00±20.81	185.70±10.23	161.10±31.86
	Phrachin Buri	155.40±16.26	154.80±12.55	162.80±22.76
	Ratchaburi	124.80±7.697	180.20±10.33	179.40±8.47
	Phetchaburi	108.20±9.42	125.20±8.36	131.40±11.89
Mean±SE		138.20±10.23 ns	169.50±13.37*	167.30±11.61*
Southern	Chumphon	146.00±15.40 ns	162.80±22.95*	183.20±8.09*
Negative control		133.40±30.12		
Positive control		269.40± 5.28		

ns = non significant from negative control

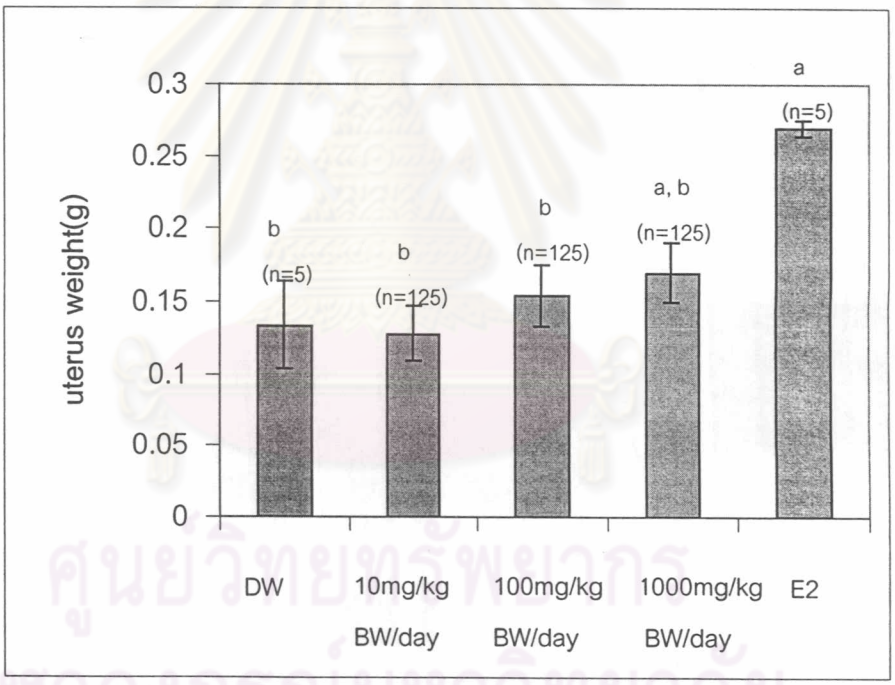

Figure 4-39. The uterine weight of rats in the control group and the treatment group.

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

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

5. Histological study

Histological study was performed under a light microscope. The adema of uterus was found in rats treated with 1,000 mg/kgBW/day of *P. mirifica* from Kanchanaburi and 17β-estradiol but no abnormalities in gross anatomy were observed in liver during necropsy (Figure 4-40).

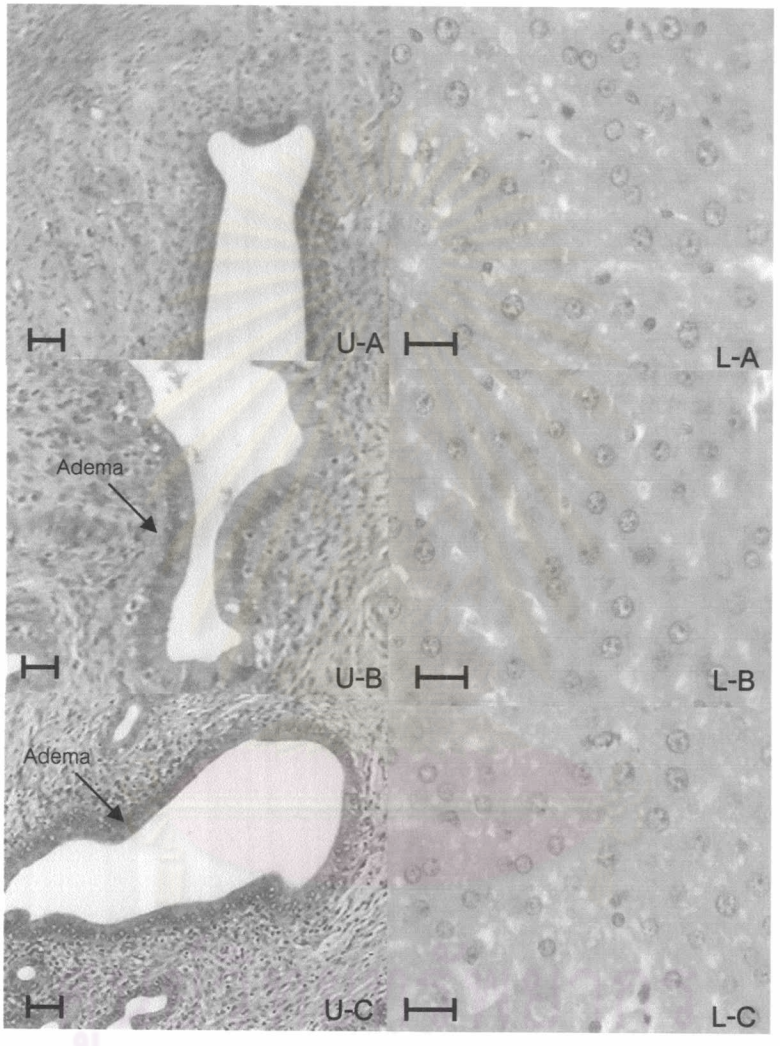

Figure 4-40. Histological study of uterus (U) and liver (L) of negative control (A), positive control (B), 1,000 mg/kg BW of *P. mirifica* from Kanchanaburi group (C) (The scale bar represented 10 μm).

6. The correlation analysis between vaginal cornification and isoflavone content of *P. mirifica*.

The result of estrogenic activity of *P. mirifica* that is determined by vaginal cornification assay and the isoflavone content (Subtang, 2002). It was found that the 7 samples of vaginal cornification, 2 highest and 2 lowest activity are correlated with the total isoflavone or individual isoflavone contents including puerarin, daidzin, genistin, daidzein and genistein ($P > 0.05$) (Table 4-5).

Table 4-5. The correlation between the estrogenic activity of *P. mirifica* sample determined by vaginal cornification assay and the isoflavone contents (Subtang, 2002).

Provinces	Rank of estrogenic activity by vaginal cornification assay	Rank of Total isoflavone	Total isoflavone (Mean±S.E.)	Puerarin (Mean±S.E.)	Daidzin (Mean±S.E.)	Genistin (Mean±S.E.)	Daidzein (Mean±S.E.)	Genistein (Mean±S.E.)
Kanchanaburi	1	1	198 ±4.6	45.25 ±1.1	50.24 ±3.23	85.69 ±1.23	13.92 ±1.26	3.19 ±0.29
Mae Hong Son	2	4	119.12 ±6.5	30.25±0.44	13.69±0.21	10.27+0.19	7.88±0.18	0.87±0.01
Phetchaburi	24	11	78.71±3.15	13.19±0.45	20.82±1.78	37.56±1.33	6.00±0.24	1.13±0.04
Uttharadith	25	15	62.96±1.03	87.05± 0.79	11.48± 0.21	14.83± 0.22	4.78± 0.37	1.42± 0.14
Correlation	None (r_s= 0.961)							

7. The correlation analysis between vaginal cornification and MCF-7 cells proliferation after *P. mirifica* treatment

The result of estrogenic activity of *P. mirifica* that is determined by vaginal cornification assay and the MCF-cells proliferation assay (Trisap, 2003). It was found that the vaginal cornification are not correlated with MCF-7 proliferation test (P> 0.05) (Table 4-6) .

Table 4-6. The correlation analysis between the estrogenic activity of *P. mirifica* sample determined by vaginal cornification assay and MCF-7 proliferation (Trisap, 2003).

Provinces	Rank of vaginal cornification	Rank of proliferation	Proliferation effect (1 μg/ml) of % control
Kanchanaburi	1	23	111.33±6.77
Mae Hong Son	2	8	127.04 ± 8.81
Phetchaburi	24	24	105.92±4.99
Uttharadith	25	19	112.92±3.77
Correlation	None (r_s=0.438)		