CHAPTER IV

RESULTS

Experiment 1: The effect of *B. superba* on sex hormone levels and reproductive organs in adult cyclic female rats.

Serum E_2 levels

Serum E_2 levels in all 5 groups were not changes throughout the study period when compared to the pre-treatment levels (day 1). Serum E_2 levels were also not significant difference among those 5 groups in each periods (Figure 3 and Table 2).

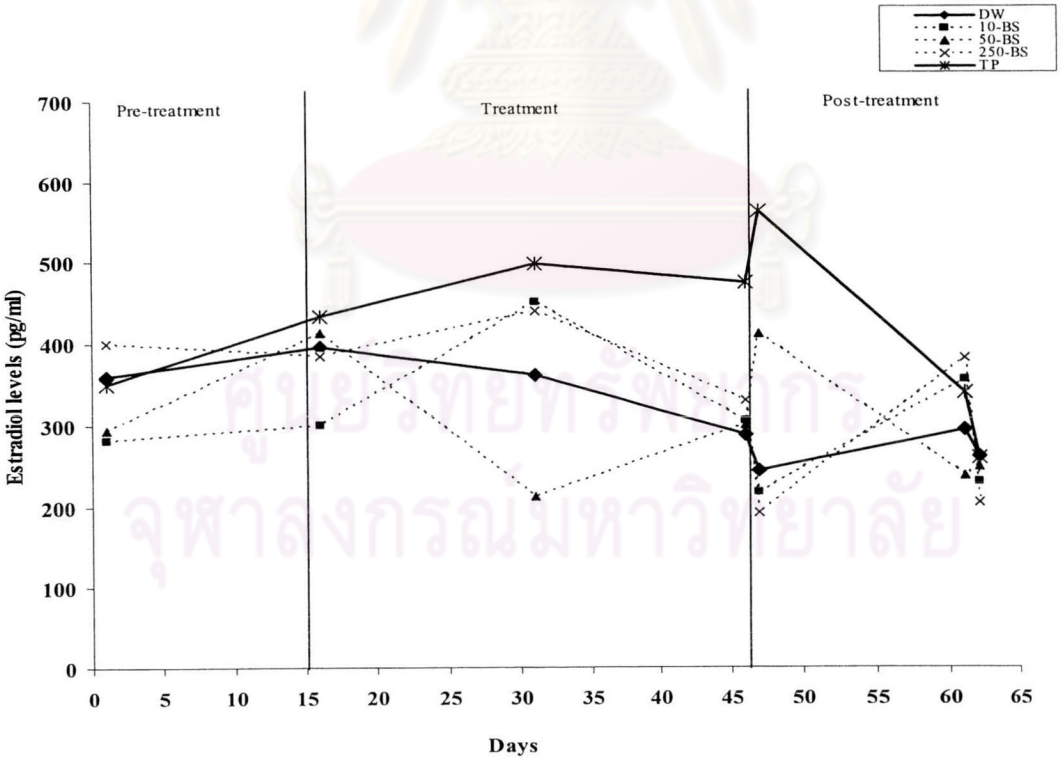


Figure 3. Serum E_2 levels in cyclic female rats treated with distilled water, *B. superba*, and testosterone propionate

Hormone levels	Treatment	Day of blood collection						
		1	16	31	46	47	61	62
	DW	359.025±54.96	396.254±46.270	360.808±52.199	287.665±39.72	243.170±18.51	294.274±102.48	260.364±74.06
E_2 levels	10-BS	281.201±47.49	299.695±49.92	450.550±98.250	304.066±36.22	216.086±28.04	354.592±166.22	229.472±81.60
(pg/ml)	50-BS	292.944±50.85	412.075±91.33	211.576±29.770	299.730±74.770	411.270±21.05	237.160±41.37	248.394±63.90
	250-BS	400.368±99.88	384.053±101.69	438.822±171.31	329.213±177.31	191.388±56.39	381.588±155.44	203.894±27.27
	TP	349.235±35.35	432.964±69.00	496.972±73.860	473.213±48.34	562.250±95.09	339.152±50.64	258.412±65.39
	DW	7.637±0.73	6.982±0.44	06.412±0.65	6.354±0.52	6.820±1.32	5.994±0.91	6.752±2.30
FSH levels	10-BS	8.216±0.96	8.199±1.77	05.161±0.49^{b2}	7.549±1.27^{b2}	7.842±0.68^{b2}	7.160±2.37^{b2}	5.798±1.25^{b2}
(ng/ml)	50-BS	8.736±1.02	8.870±2.21	10.916±4.50^{b2}	6.156±0.85^{b2}	6.754±0.49^{b2}	8.770±2.77^{b2}	6.804±0.85^{b2}
	250-BS	7.635±1.32	7.524±1.58	09.088±2.96^{b2}	8.568±2.55^{b2}	6.570±1.59^{b2}	7.862±3.78^{b2}	7.164±0.94^{b2}
	TP	9.855±1.59	10.52±2.23	00.252±0.07^{**,a2}	0.529±0.21^{**,a2}	0.384±0.13^{**,a2}	0.072±0.01^{**,a2}	0.100+0.01^{**,a2}
	DW	0.158±0.018	0.175±0.033	0.144±0.009	0.173±0.024	0.204±0.001	0.180±0.029	0.190±0.031
LH levels	10-BS	0.160±0.036	0.189±0.022	0.205±0.034^{b2}	0.197±0.031^{b2}	0.203±0.043^{b2}	0.181±0.053^{b2}	0.196±0.067^{b2}
(ng/ml)	50-BS	0.152±0.022	0.178±0.014	0.191±0.025^{b2}	0.198±0.023^{b2}	0.198±0.034^{b2}	0.184±0.018^{b2}	0.204±0.035^{b2}
	250-BS	0.145±0.010	0.140±0.041	0.154±0.036^{b2}	0.155±0.036^{b2}	0.164±0.042^{b2}	0.149±0.033^{b2}	0.146±0.005^{b2}
	TP	0.169±0.023	0.175±0.018	0.028±0.008^{**,a2}	0.038±0.011^{**,a2}	0.025±0.005^{**,a2}	0.023±0.004^{**,a2}	0.028±0.003^{**,a2}

Table 2. Serum E_2, FSH and LH levels in cyclic female rats treated with distilled water, *B. superba* and testosterone propionate

*, ** = P<0.05 and P<0.01 compared to the pre-treatment levels (day 1)

a ,a_2 = P<0.05 and P<0.01 compared to DW group

b, b_2 = P<0.05 and P<0.01 compared to TP group

Serum FSH levels

When compared to the pre-treatment levels (day 1), serum FSH levels in DW and BS groups were not changes throughout the study period, whereas the serum FSH levels were highly significantly decreased ($P<0.01$) since day 30 until the last day of post-treatment period (day 61) in TP group. Moreover, the decrease of serum FSH levels between day 31-62 in TP group was also highly significant difference from the DW and BS groups. The BS groups did not show any significant difference of serum FSH levels from the DW group in all 3 treatment periods(Figure 4 and Table 2).

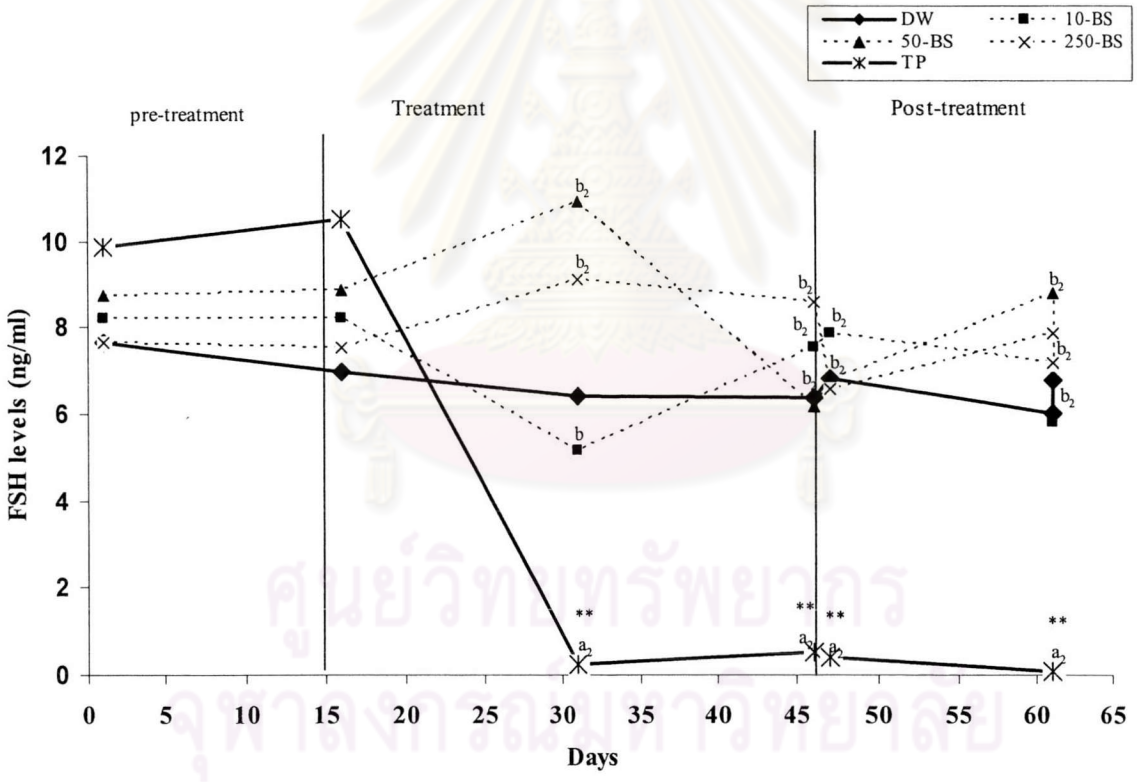


Figure 4. Serum FSH levels in cyclic female rats treated with distilled water, *B. superba*, and testosterone propionate.

** = $P<0.05$ and $P<0.01$ compared to the pre-treatment levels (day 1)

a_2 = $P<0.01$ compared to DW group

b, b_2 = $P<0.05$ and $P<0.01$ compared to TP group

Serum LH levels

The patterns of serum LH levels in all 5 groups of rats were similar to that of FSH levels (Figure 5 and Table 2).

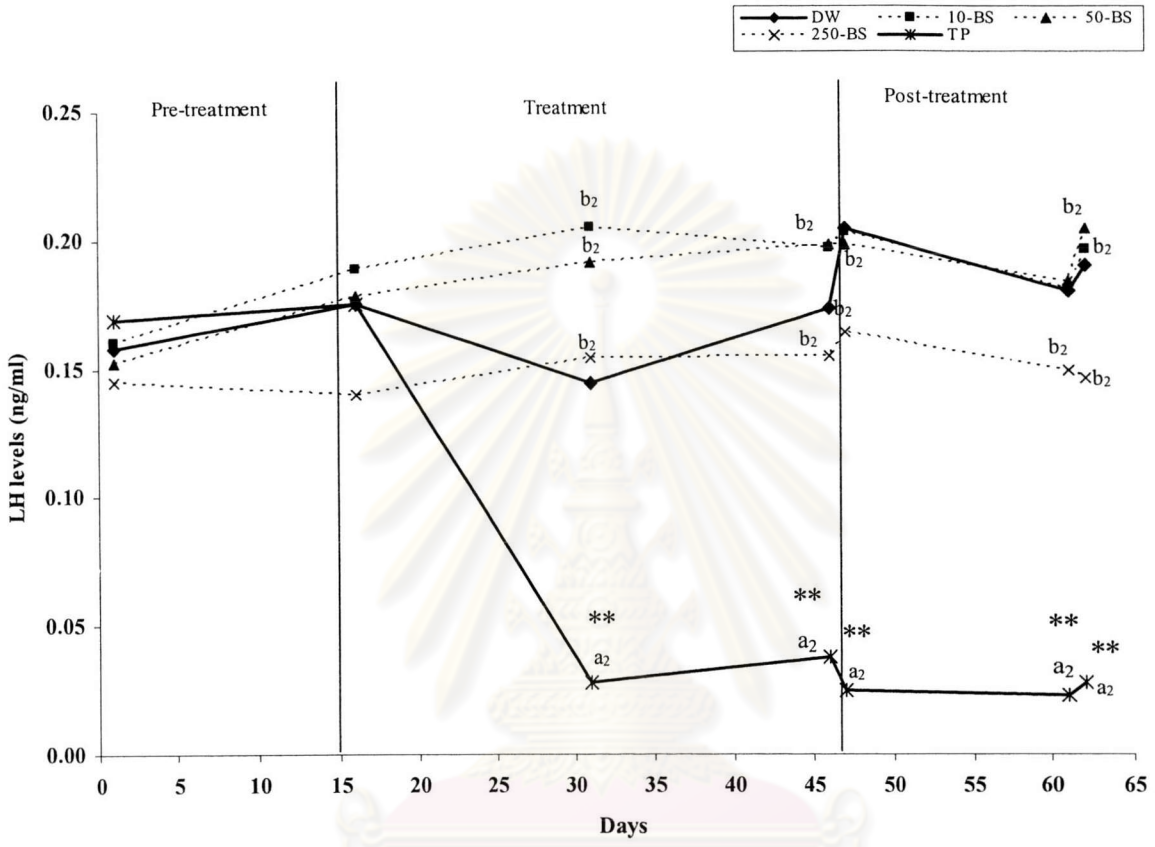


Figure 5. Serum LH levels in cyclic female rats treated with distilled water, *B. superba*, and testosterone propionate.

** = P<0.01 compared to the pre-treatment levels (day 1)

a_2 = P<0.01 compared to DW group

b_2 = P<0.01 compared to TP group

The body weights

When compared to the pre-treatment period (day 1), body weights in rat treated with DW and BS did not change throughout the study period. In contrast, body weights of TP group were significantly increased after 15 day of injection and reached the plateau at day 21 of treatment period.

When compared to the DW group, body weights in rat treated with BS did not difference throughout the study period. However, body weights in TP injected rat were significantly higher than the DW group since day 36 of study period.

When compared to the TP group, body weights in rat treated with BS did not difference throughout the study period (Figure 6).

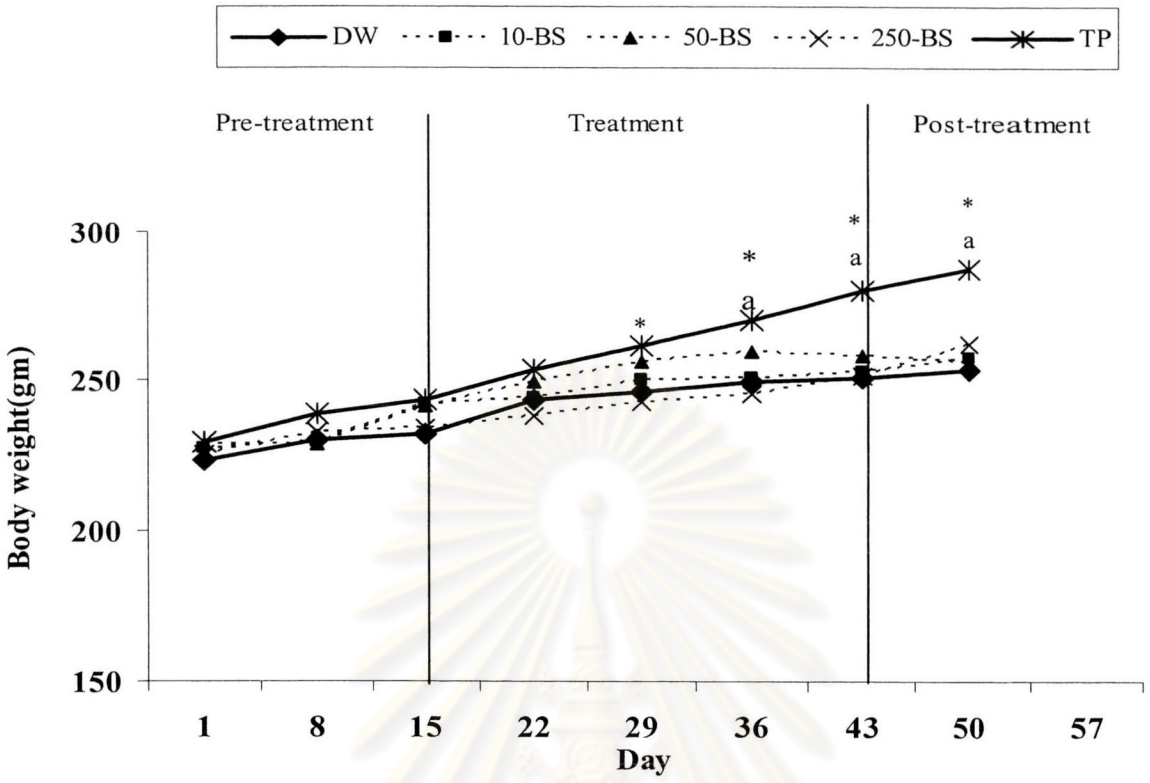


Figure 6. Body weight s in cyclic female rats treated with distilled water, *B. superba*, and testosterone propionate.

* = P<0.01 compared to the pre-treatment period (day 1)

a = P<0.01 compared to DW group

Weight and Absolute weight of reproductive organs

Weight and absolute weight of uterus

When compared the weight and the absolute weight of uterus between the end of treatment and the end of post-treatment periods, there were no different in DW and BS groups, but the weight and the absolute weight of uterus were significantly lower during the end of post-treatment period in TP group. When compared to DW group, the weight and the absolute weight of uterus at the end of treatment period were significantly higher in TP group than in DW group. The increase of the weight and the absolute weight of uterus during the end of treatment period in TP group were also significantly higher than that of the BS groups (Table 3).

Weight and absolute weight of ovary

When compared the weight and the absolute weight of ovary between the end of treatment and the end of post-treatment periods, the value was increased at the end of post-treatment period in TP group. When compared to DW group, only the value of TP group was significantly lower than the DW group in both periods. The values were also significantly lower than the BS groups in both periods. However, there were no significant differences of those values at both periods between DW and BS groups (Table 3).

	Treatment	ovary weight		absolute ovary weight (organ weight/body weight)	
		end of treatment (gm)	end of post-treatment (gm)	end of treatment $x10^{-4}$	end of post-treatment $x\ 10^{-4}$
ovary	DW	0.061±0.003	0.0616±0.004	2.36±0.125	2.445±0.177
	10-BS	0.0579 ± 0.005^{b2}	0.0572±0.003	2.183 ± 0.275^{b2}	2.205 ± 0.143^{b}
	50-BS	0.0665 ± 0.004^{b2}	0.0612±0.007	2.399 ± 0.172^{b2}	2.039 ± 0.249^{b}
	250-BS	0.0601 ± 0.008^{b2}	0.0566±0.006	2.446 ± 0.298^{b2}	2.269 ± 0.249^{b}
	TP	0.033 ± 0.003^{a2}	0.0474±0.001**	1.168 ± 0.144^{a2}	$1.57\pm0.054^{*,a}$
	Treatment	uterus weight		absolute uterus weight (organ weight/body weight)	
		end of treatment (gm)	end of post-treatment (gm)	end of treatment $x10^{-3}$	end of post-treatment $x10^{-3}$
uterus	DW	0.3288±0.026	0.322±0.031	1.27±0.096	1.277±0.12
	10-BS	0.310 ± 0.027^{b2}	0.3364±0.041	1.17 ± 0.141^{b2}	1.324±0.177
	50-BS	0.3534 ± 0.015^{b2}	0.3612±0.047	1.164 ± 0.06^{b2}	1.447±0.161
	250-BS	0.3734 ± 0.025^{b2}	0.375±0.057	1.124 ± 0.127^{b2}	1.514±0.239
	TP	0.5534 ± 0.117^{a}	0.3902±0.032*	1.936 ± 0.413^{a}	1.297±0.126*

Table 3. The weights and the absolute weights of uterus and ovary in cyclic female rats treated with distilled water, *B. superba* and testosterone propionate.

*,** = $P<0.05$ and $P<0.01$ compared to the end of treatment period

a, a_2 = $P<0.05$ and $P<0.01$ compared to DW group

b, b_2 = $P<0.01$ and $P<0.01$ compared to TP group

Estrous cycle

The rats treated with DW and BS in any dosages showed a regular estrous cycle of 4-5 days during 3 experimental periods. However, the rats treated with TP showed an unestrous cycle with the appearance of leukocyte cells throughout the treatment and post-treatment periods (Table 4 and Figure 7).

Table 4. The estrous cycle of female rats treated with distilled water, *B. superba* and testosterone propionate during 3 experimental periods.

Group	Pre-treatment (days)	Treatment (days)	Post-treatment (days)
DW	4.62±0.19	5.11±0.49	4.28±0.17
10-BS	4.64±0.2	4.85±0.39	4.02±0.18
50-BS	4.70±0.11	4.46±0.11	4.00±0.28
250-BS	4.37±0.15	4.52±0.37	4.34±0.31
TP	4.46±0.09	–	–

Figure 7. Daily monitoring of vaginal cytology from cyclic female rats treated with distilled water (A), *B. superba* at doses of 10(B), 50(C) and 250(D) mg/Kg.BW/day and testosterone propionate (E) in pre-treatment (violet stripe), treatment (light green stripe) and post-treatment (light brown stripe) periods. Numbers at the left corner represent individual animals.

■ Represented neucleated cell

▬ Represented cornified cell

□ Represented leukocyte cell

Figure 7. Daily monitoring of vaginal cytology from cyclic female rats treated with distilled water (A), *B. superba* at doses of 10(B), 50(C) and 250(D) mg/Kg.BW/day and testosterone propionate (E) in pre-treatment (violet stripe), treatment (light green stripe) and post-treatment (light brown stripe) periods. Numbers at the left corner represent individual animals.

■ Represented neucleated cell

▬ Represented cornified cell

□ Represented leukocyte cell

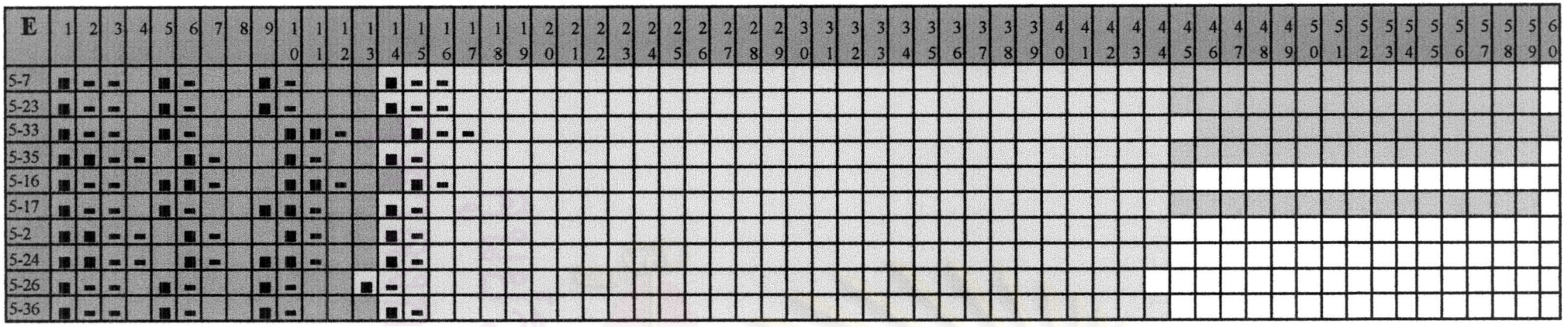


Figure 7. Daily monitoring of vaginal cytology from cyclic female rats treated with distilled water (A), *B. superba* at doses of 10(B), 50(C) and 250(D) mg/Kg.BW/day and testosterone propionate (E) in pre-treatment (violet stripe), treatment (light green stripe) and post-treatment (light brown stripe) periods. Numbers at the left corner represent individual animals.

■ Represented neucleated cell

▬ Represented cornified cell

□ Represented leukocyte cell

Histological study of reproductive organs

Histology of ovary

The histological study of rat ovary in DW, BS and TP groups showed the normal structure and numerous of primary follicle, secondary follicle and Graafian follicle. There were no differences between the structure and number of primary follicle at the end of treatment and the end of post-treatment period in each group. The ovarian tissues of rat in BS groups were not difference from DW and TP groups (Figure 8-10).

Histology of uterus

Histology of rat uterus composed of endometrium, myometrium and perimetrium layer. Endometrium of DW showed a simple columnar epithelium and tubular endometrial glands or uterine gland that open into the lumen of uterus (Figure 11). There were no differences of histological structure between uterus at the end of treatment and the end of post-treatment period. The histological study of rat uterus in 10 and 50-BS groups at the end of treatment period also showed a simple columnar epithelium, but the numbers of uterine glands were decreased. In contrast, the simple columnar epithelium and the number of uterine glands were increased in 250-BS group compared to the DW group (Figure12). However, it could recover at the end of post-treatment period (Figure 13). In TP group, at the end of treatment period, the uterine gland was decreased when compared to the DW group, and recover at the end of post-treatment period (Figure 13). The histological study of uterus in BS groups and TP groups showed that the numbers of tubular endometrial

glands were lower than that of DW group. Except in 250-BS group, the numbers were higher than the DW group

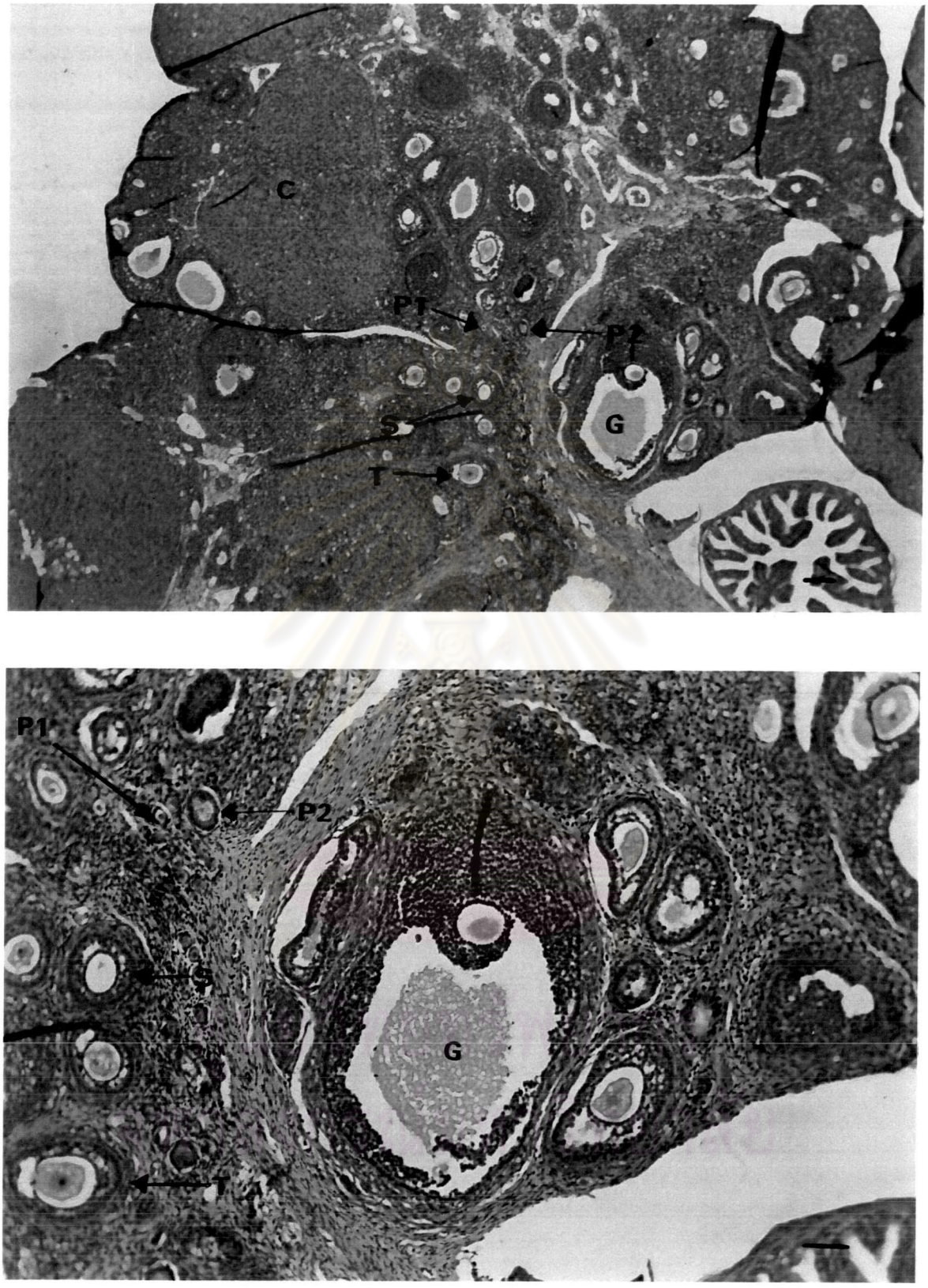
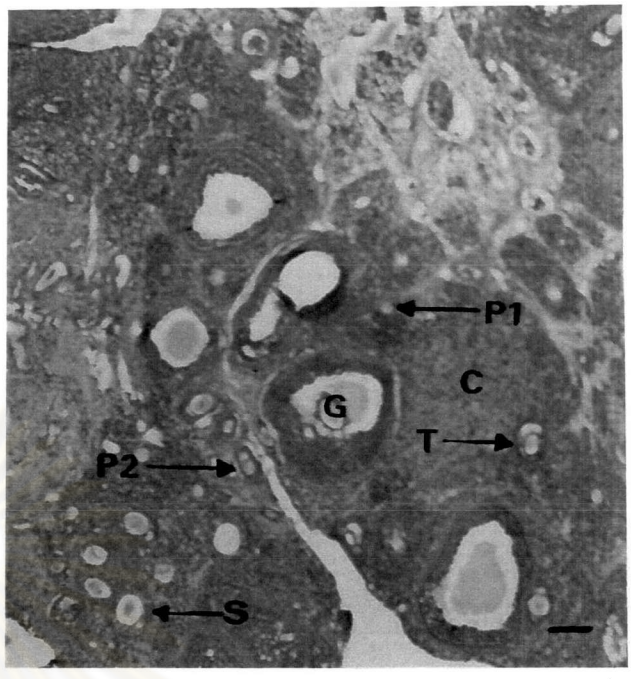
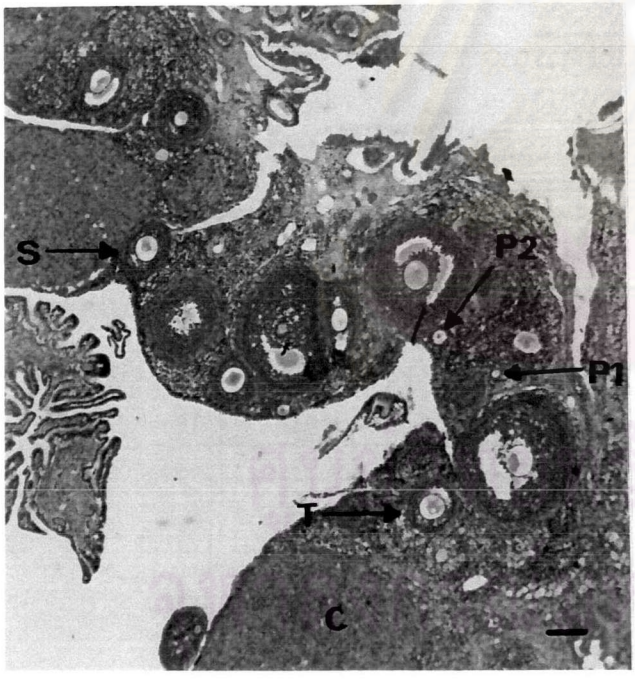


Figure 8. Ovarian morphology in DW group of cyclic female rats at the end of treatment period. P1=Primodial follicle, P2=Primary follicle, S=Secondary follicle, G=Graafian follicle and C=Corpus luteum. H&E stain. (Scale bars=50 μm)

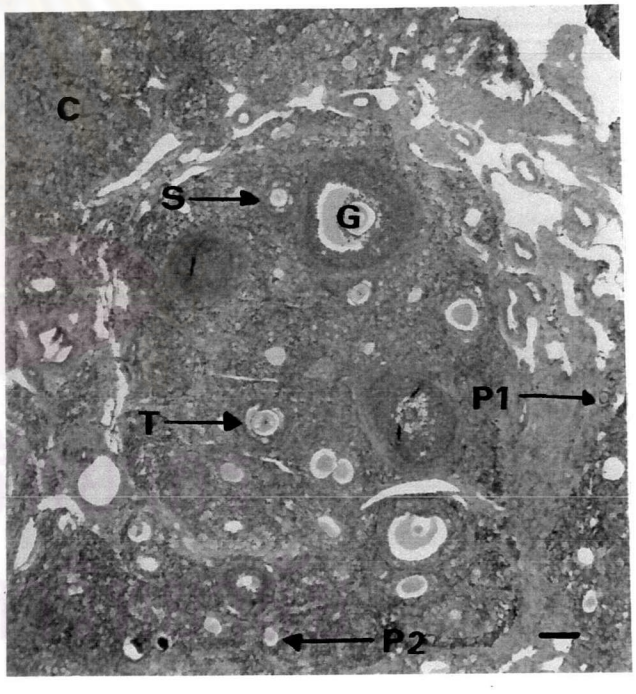
10-BS



50-BS



250-BS



TP

Figure 9. Ovarian morphology in cyclic female rats treated with 10-BS, 50-BS, 250-BS and TP at the end of treatment period. P1=Primodial follicle, P2=Primary follicle, S=Secondary follicle, G=Graafian follicle and C=Corpus luteum. H&E stain. (Scale bars=50 μm)

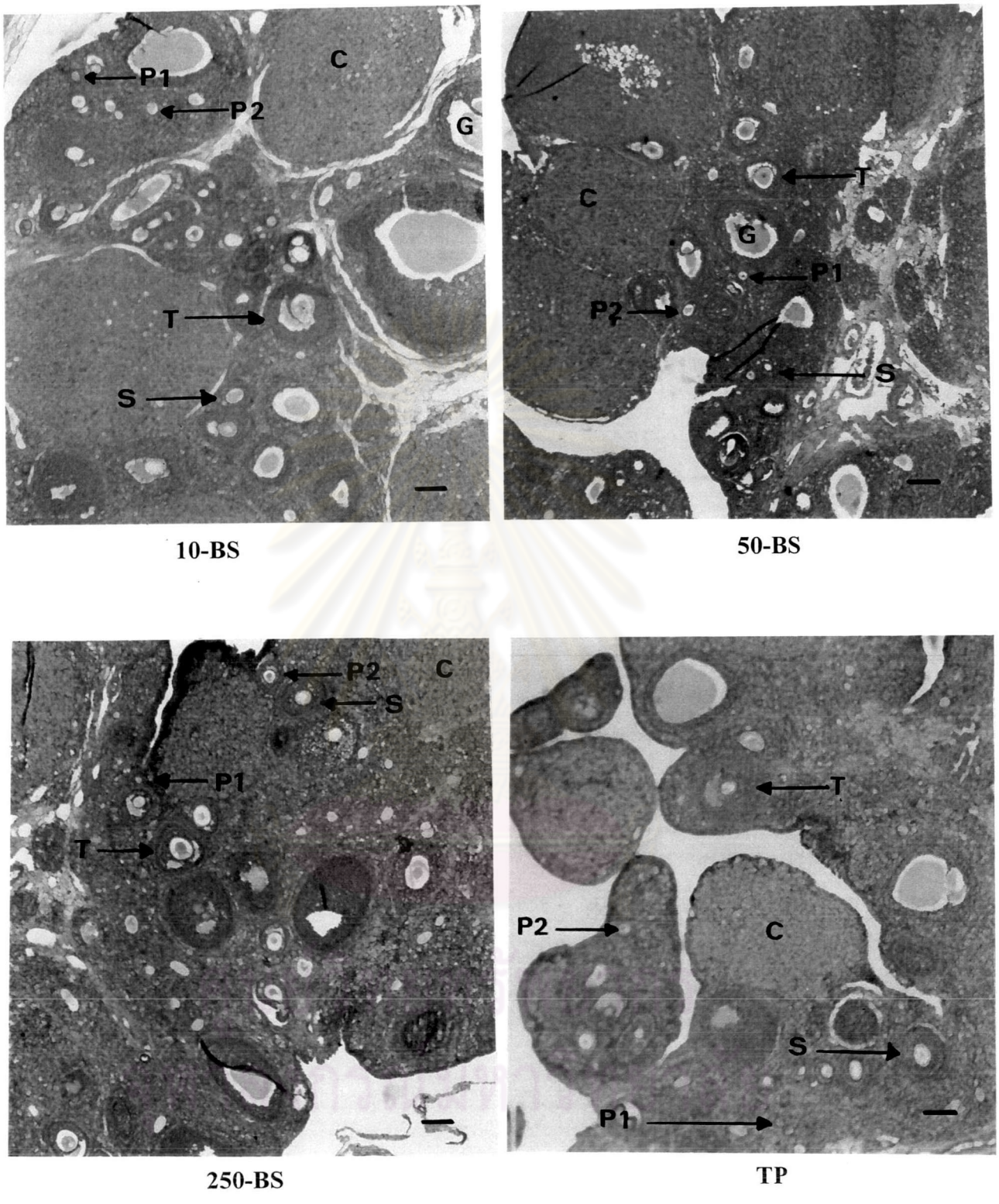


Figure 10. Ovarian morphology in cyclic female rats treated with 10-BS, 50-BS, 250-BS and TP at the end of post-treatment period. P1=Primodial follicle, P2=Primary follicle, S=Secondary follicle, G=Graafian follicle and C=Corpus luteum. H&E stain. (Scale bars=50 μm)

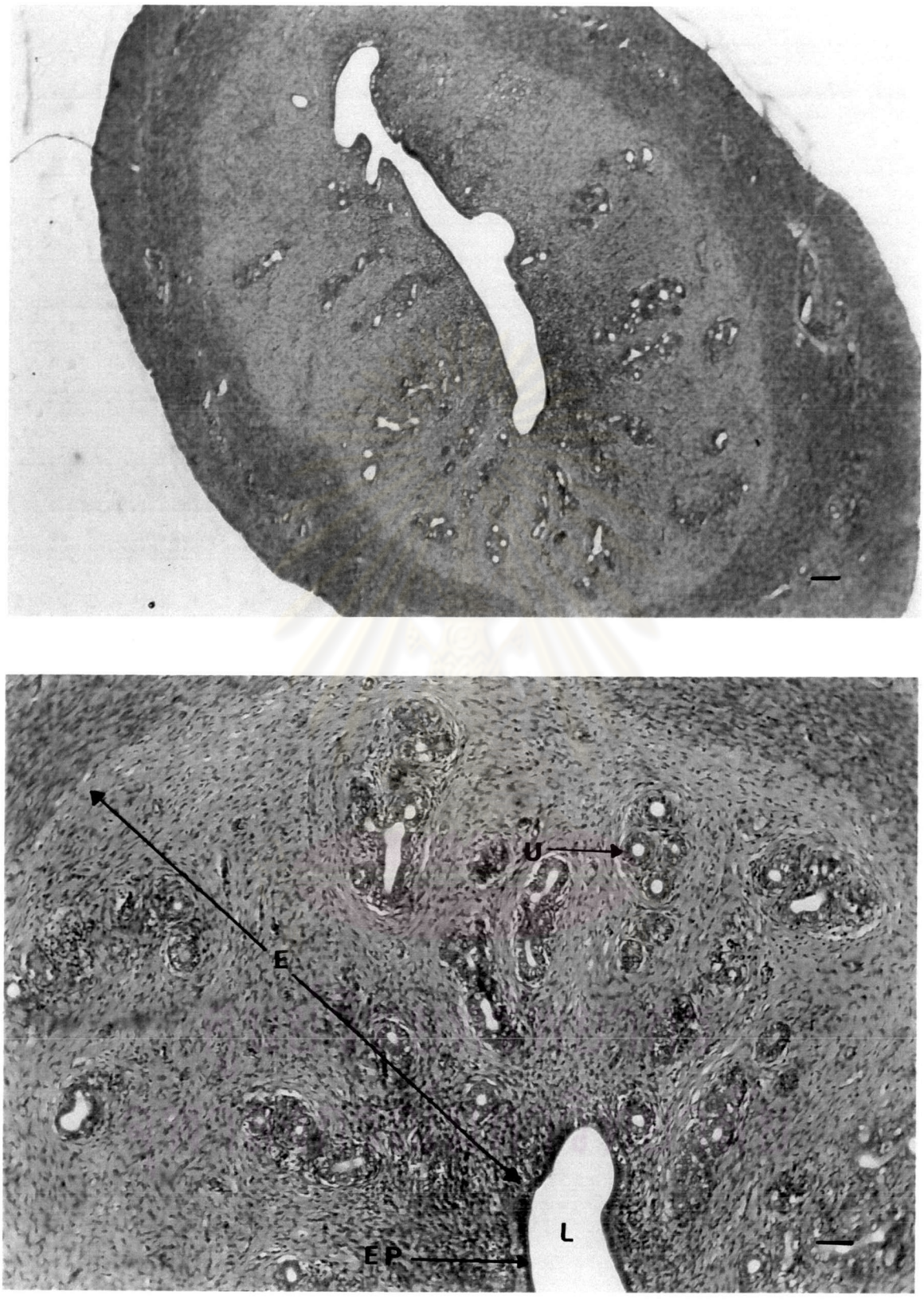


Figure 11. Uterus morphology in DW group of cyclic female rats at the end of treatment period. EP=Epithelial cell, E=Endometrium, L=Uterine lumen and U=Uterine gland. H&E stain. (Scale bars=50 μm)

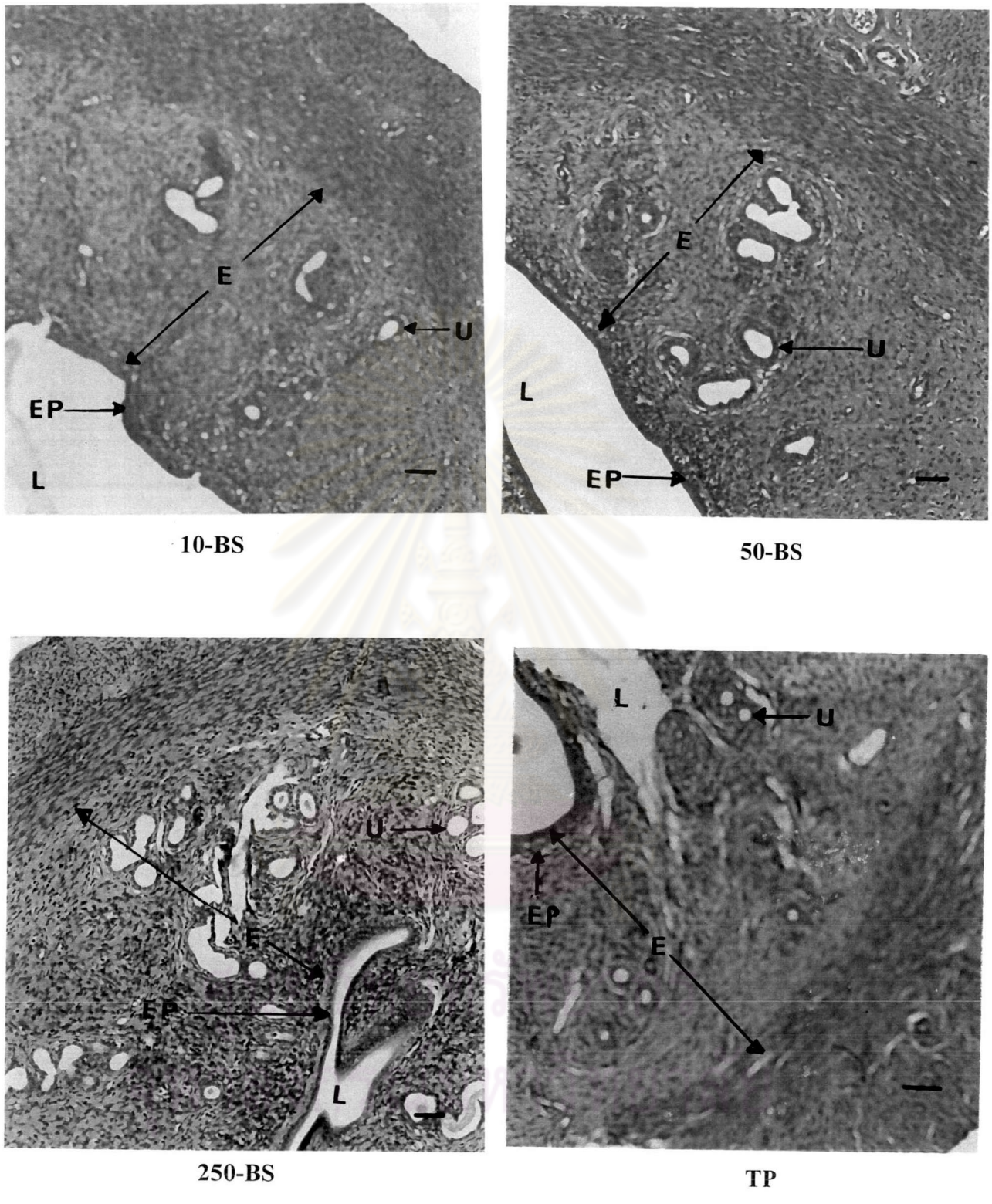


Figure 12. Uterus morphology in cyclic female rats treated with 10-BS, 50-BS, 250-BS and TP at the end of treatment period. EP=Epithelial cell, E=Endometrium, L=Uterine lumen and U=Uterine gland. H&E stain. (Scale bars=50 μm)

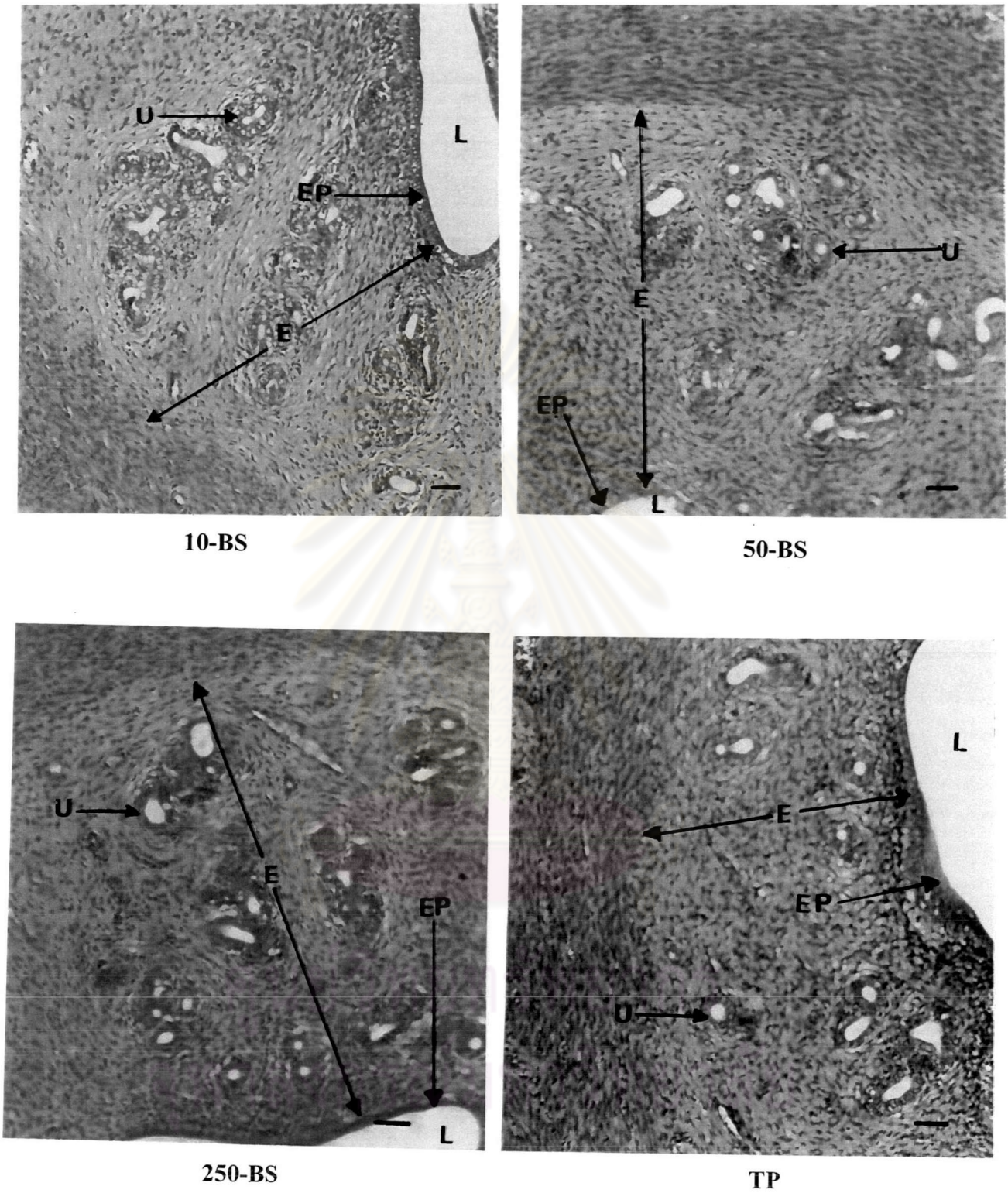


Figure 13. Uterus morphology in cyclic female rats treated with 10-BS, 50-BS, 250-BS and TP at the end of post-treatment period. EP=Epithelial cell, E=Endometrium, L=Uterine lumen and U=Uterine gland. H&E stain. (Scale bars=50 μm)

Experiment 2: The effect of *B. superba* on sex hormone levels and reproductive organs in ovariectomized rats.

Serum E_2 levels

Comparison serum E_2 levels between day -14 (before ovariectomy) and day 1(14 days after ovariectomy) in all 5 groups, no differences were found. When compared to the pre-treatment levels (day 1), serum E_2 levels in DW and BS groups were not changed throughout the study period. But serum E_2 levels were highly significantly increased at the last day of treatment (day 46) and the first day of post-treatment period in TP group (day 47). The increase of E_2 levels in TP group was significantly decreased to the pre-treatment levels in post-treatment period (day 61, 62). The BS groups did not show any significantly different of serum E_2 level from the DW group. But in TP group at day 47, serum E_2 levels were significantly increased at day 46 and day 47 when compared to the DW group (Figure 14 and Table 5).

Hormone levels	Treatment	Day of blood collection							
		-14	1	16	31	46	47	61	62
	DW	145.074±9.65	144.509±8.65	134.632±12.19	142.321±5.95	126.467±7.79	133.178±18.16	145.514±12.79	130.052±15.58
E_2	10-BS	152.105±14.50	120.861±5.73	129.620±9.00	139.378±9.99	125.112±7.51^{b2}	114.758±13.70^{b2}	133.282±16.42	127.025±21.16
(pg/ml)	50-BS	163.325±24.73	131.740±20.67	145.547±15.99	153.457±8.08	147.073±9.92^{b2}	146.434±14.96^{b2}	141.402±8.19	133.590±30.74
	250-BS	145.005±15.25	148.152±22.20	124.158±13.87	154.490±41.21	098.698±13.52^{b2}	097.294±26.25^{b2}	121.364±19.10	122.726±15.90
	TP	166.421±14.13	128.878±19.89	149.787±15.13	180.162±24.97	233.763±23.60^{**,a}	298.806±78.06^{**,a}	151.964±36.56	198.790±20.81
	DW	1.434±0.19	21.085±1.53	28.133±2.59	36.033±2.63**	35.905±2.28**	39.344±6.60**	40.535±3.65^{**,b2}	40.292±5.35**
FSH	10-BS	2.184±0.45	20.391±2.40	31.390±2.63	41.522±1.87^{**,b2}	42.768±3.49^{**,b2}	42.792±4.18^{**,b2}	44.907±5.86^{**,b2}	41.787±4.93^{**,b2}
(ng/ml)	50-BS	1.505±0.12	21.666±1.73	26.117±1.96	31.078±3.00^{**,b2}	37.116±3.50^{**,b2}	37.793±6.07^{**,b2}	29.465±4.42^{**,b2}	29.902±3.59^{**,b2}
	250-BS	1.875±0.20	20.327±2.28	28.217±2.94	34.646±3.01^{**,b2}	43.965±6.96^{**,b2}	33.796±4.92^{**,b2}	41.947±2.80^{**,b2}	37.157±2.77^{**,b2}
	TP	1.852±0.58	20.075±6.35	31.011±9.81	10.774±3.41^{**,a2}	05.796±1.83^{**,a2}	04.112±0.25^{**,a2}	09.812±2.93^{**,a2}	09.109±3.02^{**,a2}
	DW	0.381±3.23	17.727±3.29	27.829±4.49	36.179±8.49*	48.009±18.52**	74.580±30.14**	39.754±9.37*	35.769±7.55*
LH	10-BS	0.421±0.07	14.655±3.58	29.317±3.11	42.707±7.97^{*,b}	37.234±7.13^{*,b}	61.565±7.92^{*,b}	51.891±11.60^{*,b2}	56.027±15.32^{*,b2}
(ng/ml)	50-BS	0.411±0.10	17.936±3.00	56.334±13.83	96.965±23.82^{**,a2,b2}	94.325±23.09^{**,a2,b2}	70.851±14.26^{**,b2}	93.774±9.27^{**,a2,b2}	64.512±6.13^{**,b2}
	250-BS	0.417±0.04	18.981±5.02	27.094±3.20	73.764±22.44^{**,a2,b2}	54.040±11.47^{*,b2}	52.244±8.59^{*,b2}	33.214±8.92^{b2}	34.998±10.97^{b2}
	TP	0.409±0.10	15.285±1.59	31.336±4.31	00.118±0.04^{**,a2}	00.008±0.004^{**,a2}	00.038±0.02^{**,a2}	01.002±0.83^{**,a2}	00.755±0.60^{**,a2}

Table 5. Serum E_2, FSH and LH levels in ovariectomized rats treated with distilled water, *B. superba* and testosterone propionate

*, ** =P<0.05 and P<0.01 compared to the pre-treatment levels (day 1)

a ,a_2= P<0.05 and P<0.01 compared to DW group

b, b_2= P<0.05 and P<0.01 compared to TP group

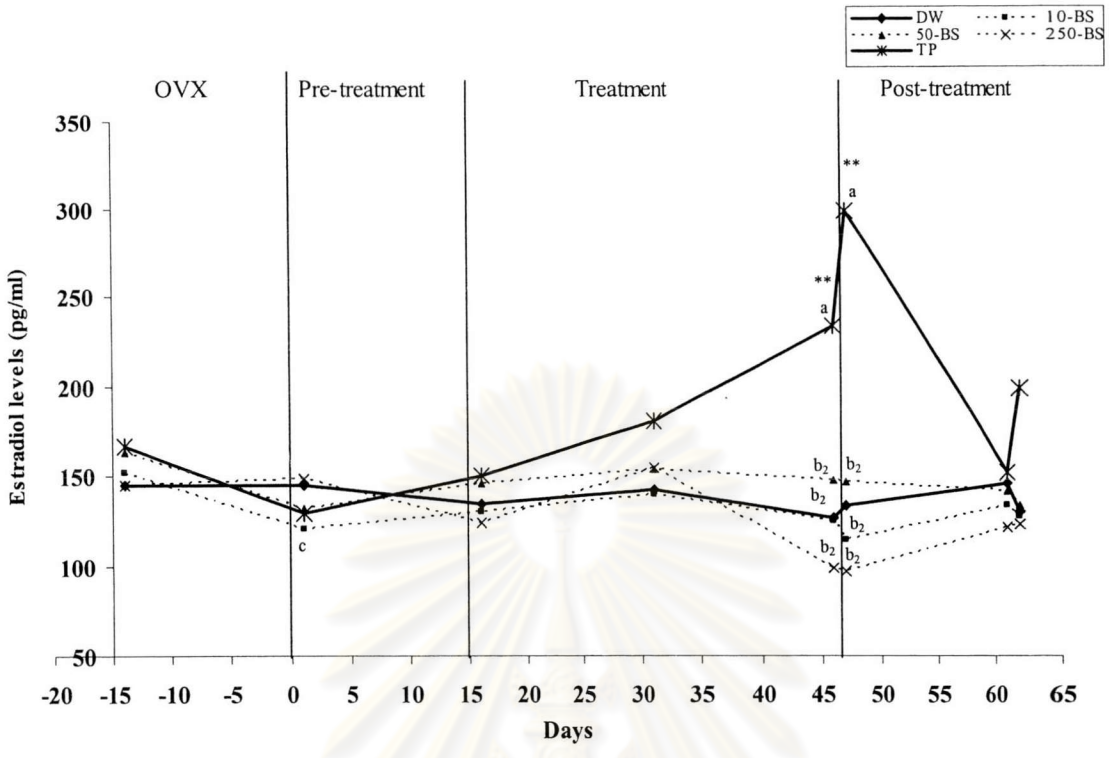


Figure 14. Serum E_2 levels in ovariectomized rats treated with distilled water, *B. superba*, and testosterone propionate.

** = P<0.01 compared to the pre-treatment levels (day 1)

a = P<0.05 compared to DW group

b_2 = P<0.01 compared to TP group

Serum FSH levels

Serum FSH levels in all 5 groups were significantly increased after ovariectomy for 14 days (1.77 ±0.19 ng/ml at day -14 and 20.7±1.53 ng/ml at day 1, respectively). When compared to the pre-treatment levels (day 1), serum FSH levels in DW and BS groups were abruptly increased from day 16-day 31 and reached a plateau thereafter. But in the TP group, FSH levels were significantly decreased since day 31 until the last day of study period (day 62). The BS groups did not show any significantly difference of serum FSH levels from the DW group in all 3 treatments. But, the decrease of serum FSH levels

in TP group between day 31-62 was highly significant difference (P<0.01) from the DW group (Figure 15 and Table 5).

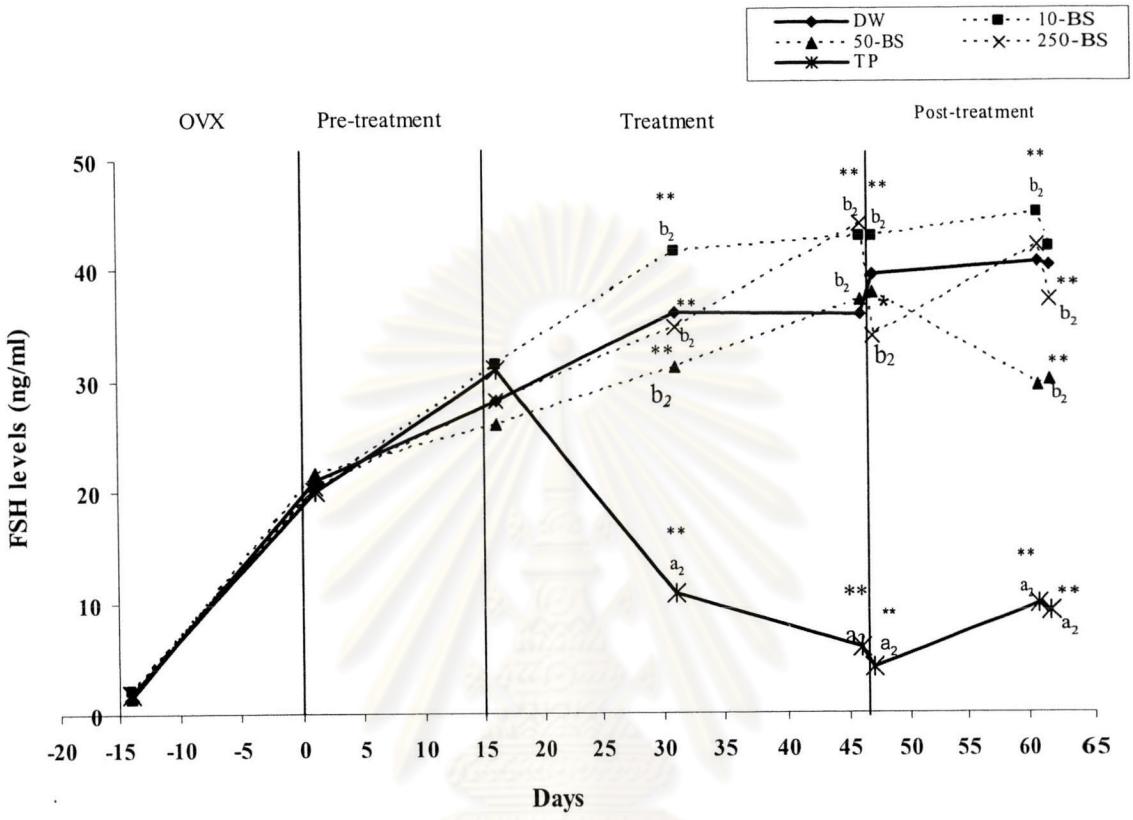


Figure 15. Serum FSH levels in ovariectomized rats treated with distilled water, *B.superba*, and testosterone propionate.

*, ** = P<0.05 and P<0.01 compared to the pre-treatment levels (day 1)

a_2 = P<0.01 compared to DW group

b_2 = P<0.01 compared to TP group

Serum LH levels

Serum LH levels in all 5 groups were significantly increased after ovariecftomy for 14 day. When compared to the pre-treatment levels (day 1), serum LH levels in DW and BS groups were significantly increased since day 31 and reached a plateau thereafter. When compared to the DW group, serum LH levels were significantly higher than that of

DW group at day 31, 46 and 61 in 50-BS group and at day 31 in 250-BS group. In contrast, serum LH levels in TP group were significantly lower than the pre-treatment level (day 1) and the DW group at day 31 - day 62. The LH levels in those days of TP group were not different from day -14 or the ovariectomized day (Figure 16 and Table 5).

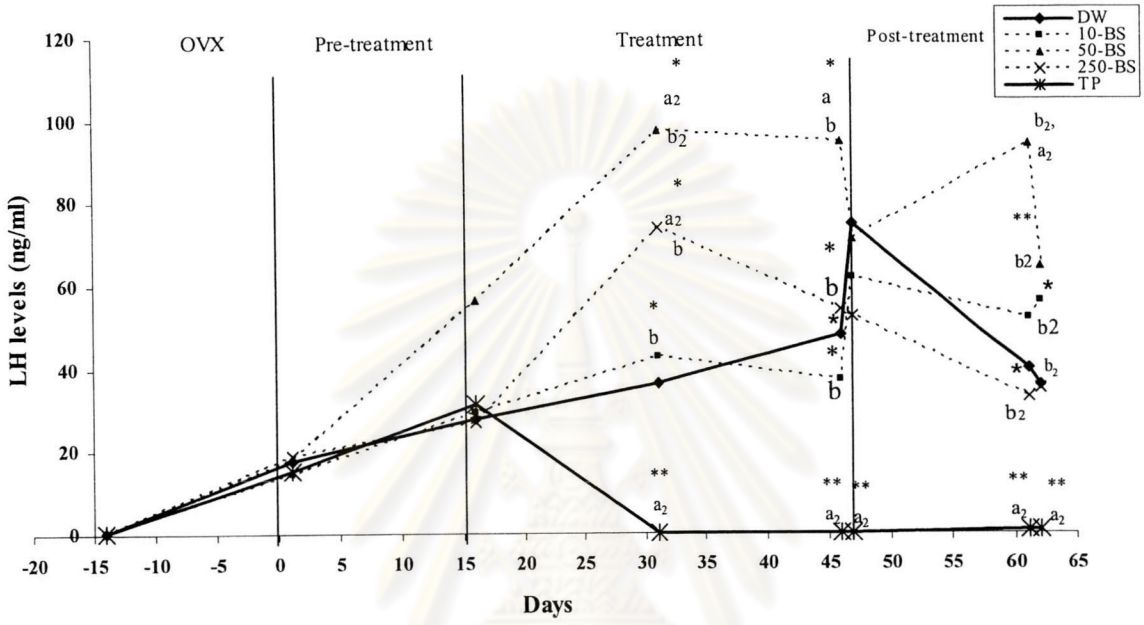


Figure 16. Serum LH levels in ovariectomized rats treated with distilled water, *B. superba*, and testosterone propionate.

*, ** = P<0.05 and P<0.01 compared to the pre-treatment levels (day 1)

a_1, a_2 = P<0.05 and P<0.01 compared to DW group

b, b_2 = P<0.05 and P<0.01 compared to TP group

The body weights

When compared to the pre-treatment period (day 1), body weights in rats treated with DW, BS and TP were significantly increased throughout the study period.

When compared to the DW group, body weights in rats treated with BS and TP did not difference throughout the study period, and body weights of rats in BS groups were also not significant difference from the TP group (Figure 17).

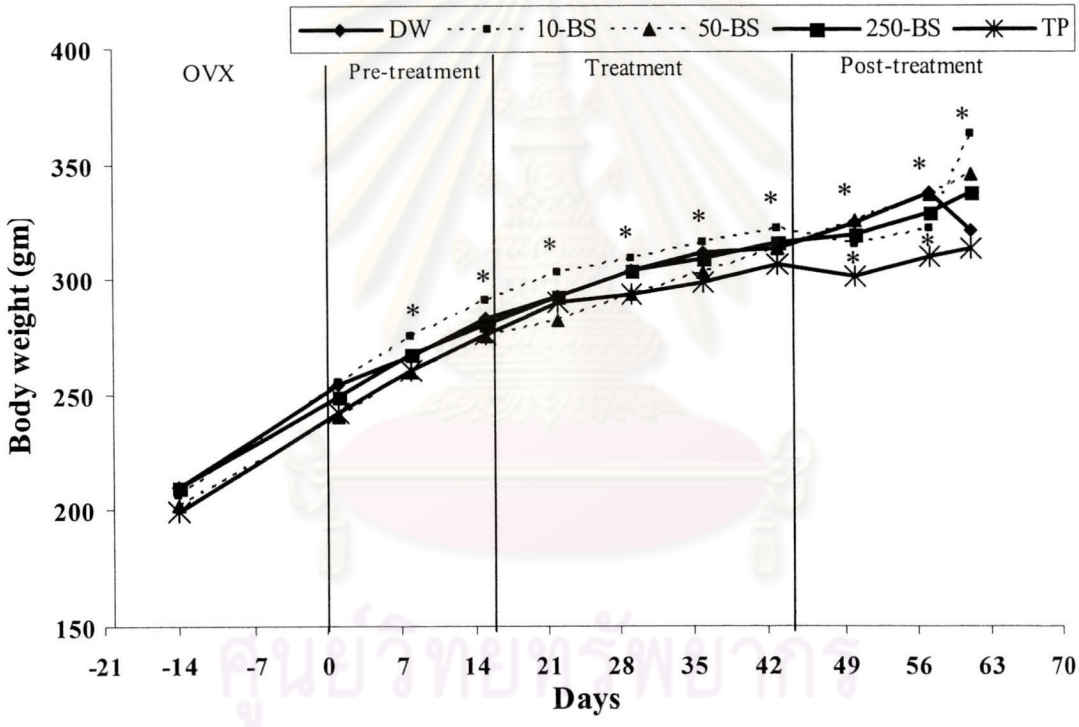


Figure 17. Body weights in ovariectomized rats treated with distilled water, *B. superba*, and testosterone propionate.

* = P<0.05 compared to the pre-treatment levels (day 1)

Weight and absolute weight of uterus

When compared the weight and the absolute weight of uterus between the end of treatment and the end of post-treatment periods, there were no significantly differences in DW and BS groups, but the weight and the absolute weight of uterus during the end of treatment period were significant higher than the end of post-treatment period in TP group. However, the lowered weights during the end of post-treatment period in TP group were still higher than the DW and BS groups. (Table 6).

	treatment	uterus weight		absolute uterus weight (uterus weight/body weight)	
		end of treatment (gm)	end of post-treatment (gm)	end of treatment ($x10^{-4}$)	end of post-treatment ($x10^{-4}$)
uterus	DW	0.106±0.004	0.092±0.01	3.342±0.087	3.27±0.642
	10-BS	0.108 ± 0.005^{b2}	0.099 ± 0.009^{b2}	3.189 ± 0.112^{b2}	3.037 ± 0.338^{b2}
	50-BS	0.103 ± 0.006^{b2}	0.095 ± 0.006^{b2}	3.156 ± 0.194^{b2}	2.733 ± 0.230^{b2}
	250-BS	0.109 ± 0.004^{b2}	0.097 ± 0.014^{b2}	3.708 ± 0.319^{b2}	2.925 ± 0.427^{b2}
	TP	0.562 ± 0.022^{a2}	$0.302\pm0.052^{a2,**}$	18.317 ± 0.822^{a2}	$9.679\pm1.695^{a2,**}$

Table 6. The weight and the absolute weight of uterus in ovariectomized rats treated with distilled water, *B. superba* and testosterone propionate.

** = $P<0.01$ compared to the end of treatment period

a_2 = $P<0.01$ compared to DW group

b_2 = $P<0.01$ compared to TP group

Histological study of reproductive organs

Histology of uterus

Complete ovariectomized rat showed the size of uterus smaller than that of the cyclic rat. The histology of uterus in DW and 10-BS groups at the end of treatment period still showed the thin endometrial mucosa layers. The mucosa epithelial cell lining was a layer of cuboidal cell and it had the low height of cell lining. The number and diameter of uterine glands were also decreased (Figure18). But in the 50 and 250-BS groups, the endometrium were thicker than those of DW and 10-BS groups. The number and diameter of uterine glands in 50-BS group were not different from the DW group, but those parameters were increased in 250-BS group. The size of uterus in TP group was bigger than the DW and BS groups. The endometrium of TP group showed the increase in epithelium cell lining, and the number and diameter of uterine glands were lower than that of DW group (Figure 19).

The histology of uterus in DW, both BS and TP groups at the end of post-treatment period showed the same changes as it appeared on the end of treatment period. No differences between the end of treatment and the end of post-treatment period were found in all groups. However, the number and diameter of uterine glands of 250-BS and TP groups at the end of post-treatment period were lower than that of the end of treatment period. The TP group also showed the decrease in epithelial cell lining and uterine size at the end of post-treatment period when compared to the end of treatment period.

Estrous cycle

The ovariectomized rats treated with DW, all BS and TP showed an unestrous cycle with the appearance of leukocyte cells throughout the treatment and post-treatment periods (Figure 21).

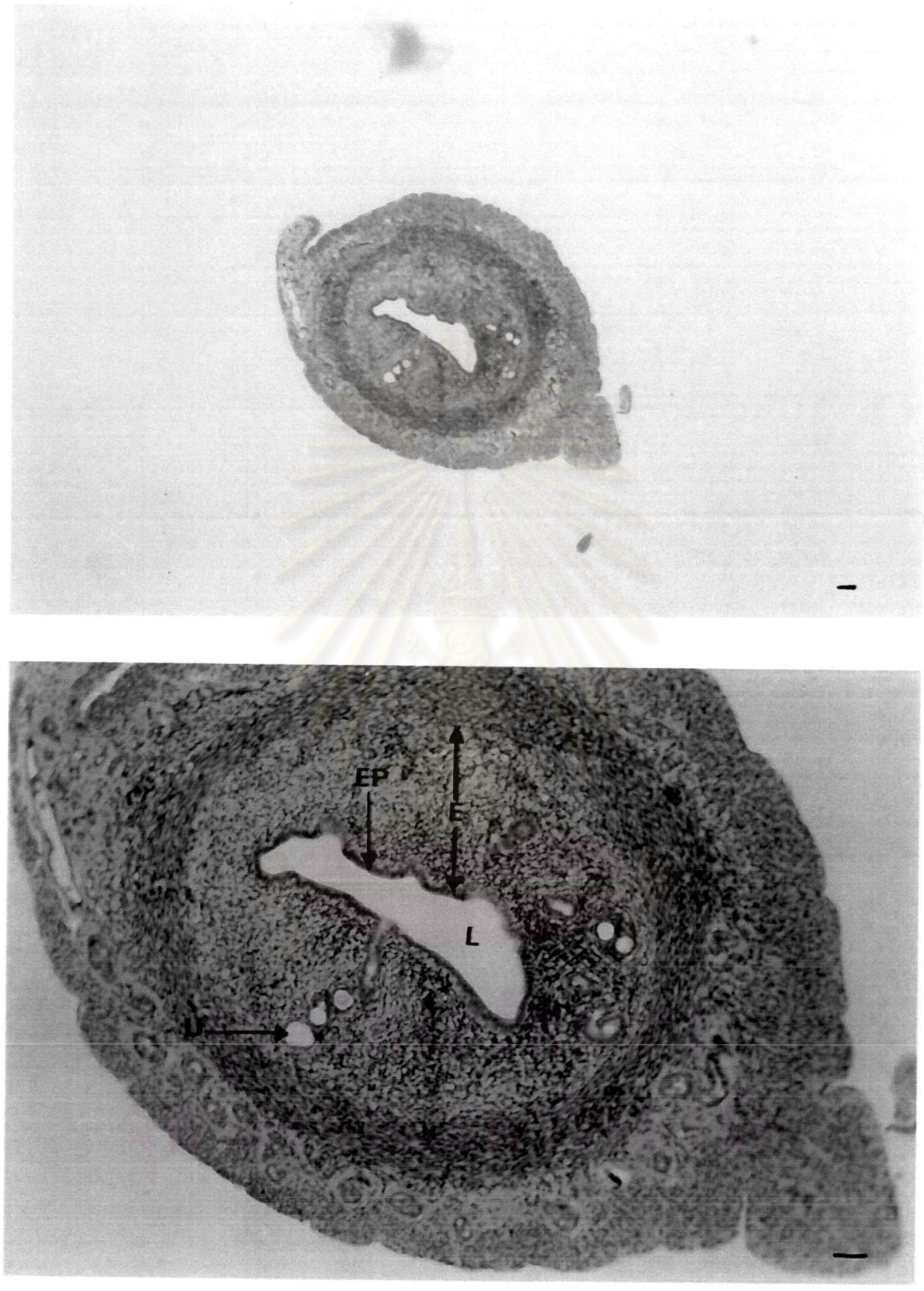


Figure 18. Uterus morphology in DW of ovariectomized rats at the end of treatment period. EP=Epithelial cell, E=Endometrium, L=Uterine lumen and U=Uterine gland. H&E stain. (Scale bars=50 μm)

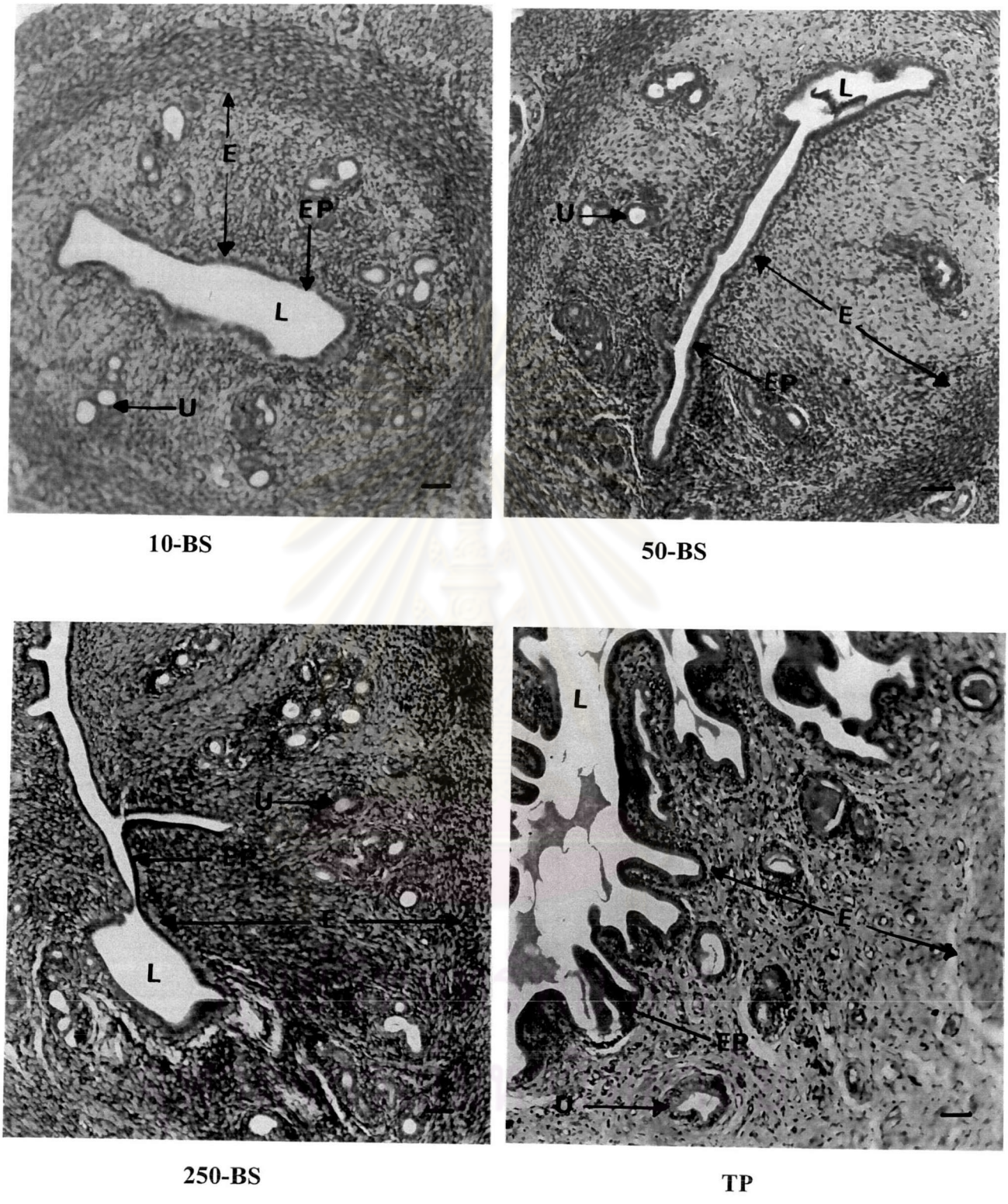


Figure 19. Uterus morphology in ovariectomized rats treated with 10-BS, 50-BS, 250-BS and TP at the end of treatment period EP=Epithelial cell, E=Endometrium, L=Uterine lumen and U=Uterine gland. H&E stain. (Scale bars=50 μm)

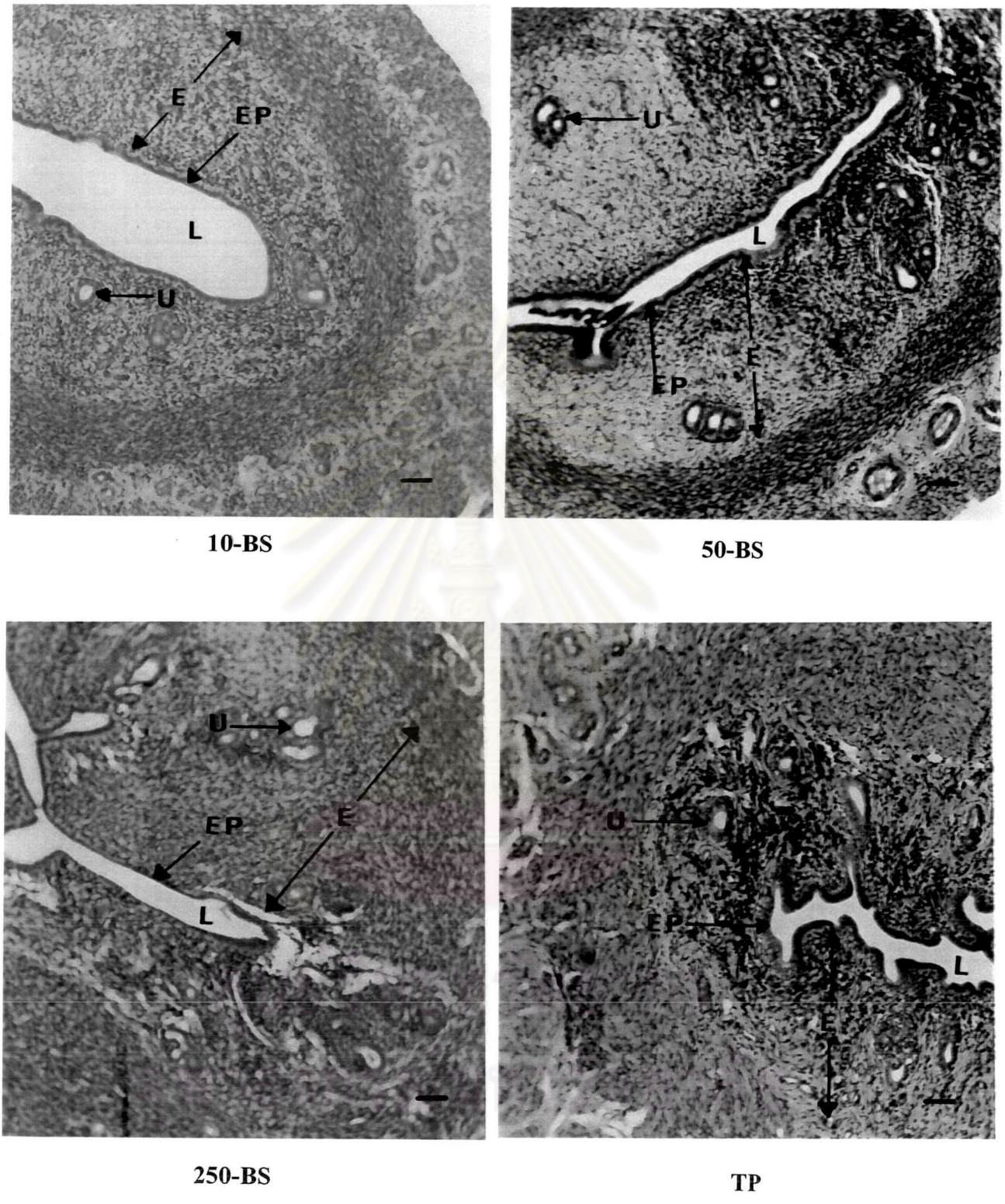


Figure 20. Uterus morphology in ovariectomized rats treated with 10-BS, 50-BS, 250-BS and TP at the end of post-treatment period. EP=Epithelial cell, E=Endometrium, L=Uterine lumen and U=Uterine gland. H&E stain. (Scale bars=50 μm)

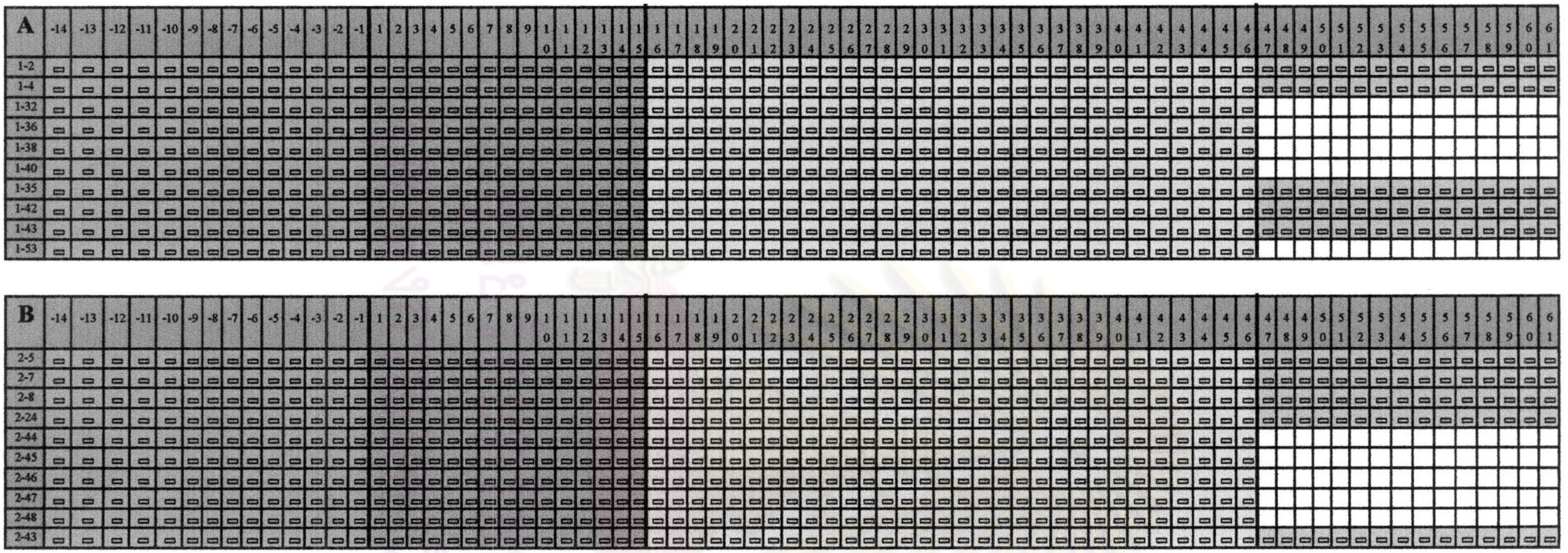


Figure 21. Daily monitoring of vaginal cytology from ovariectomized rats treated with distilled water (A), *B. superba* at doses of 10(B), 50(C), and 250(D) mg/Kg.BW/day and TP (E) in wash-out (pink stripe), pre-treatment (violet stripe), treatment (light green stripe) and post-treatment (light brown stripe) periods. Numbers at the left corner represent individual animals.

▭ Represented leukocytes cell

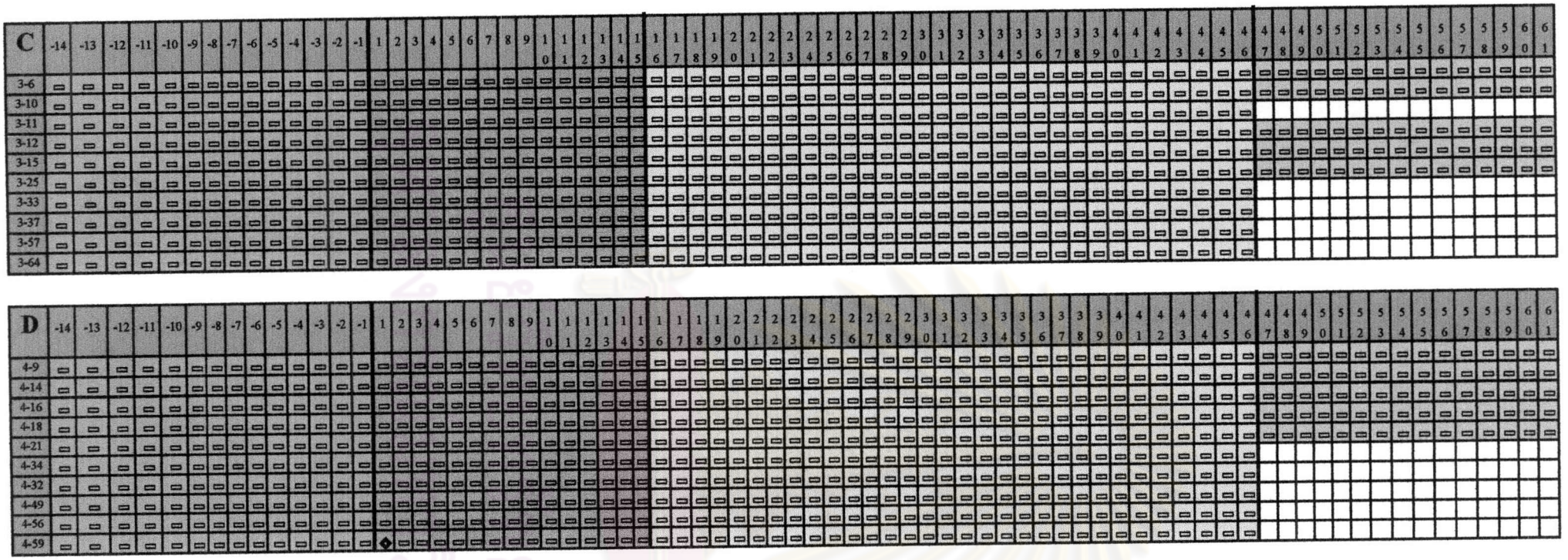


Figure 21. Daily monitoring of vaginal cytology from ovariectomized rats treated with distilled water (A), *B. superba* at doses of 10(B), 50(C), and 250(D) mg/Kg.BW/day and TP (E) in wash-out (pink stripe), pre-treatment (violet stripe), treatment (light green stripe) and post-treatment (light brown stripe) periods. Numbers at the left corner represent individual animals.

▭ Represented leukocytes cell

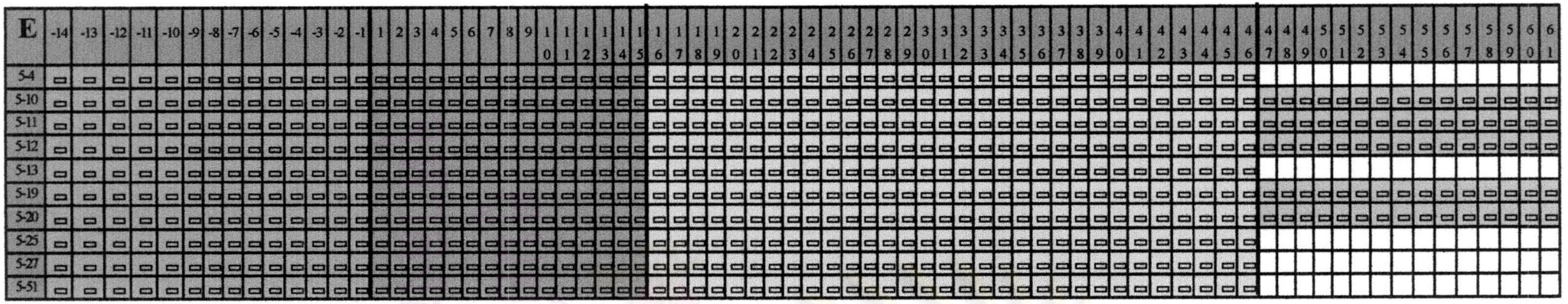


Figure 21. Daily monitoring of vaginal cytology from ovariectomized rats treated with distilled water (A), *B. superba* at doses of 10(B), 50(C), and 250(D) mg/Kg.BW/day and TP (E) in wash-out (pink stripe), pre-treatment (violet stripe), treatment (light green stripe) and post-treatment (light brown stripe) periods. Numbers at the left corner represent individual animals.

▭ Represented leukocytes cell

Experiment 3: The effect of *B. superba* on sex hormone levels and reproductive organs in normal male rats.

Serum T levels

When compared to the pre-treatment period (day 1), serum T levels in DW groups were not changes throughout the study period. Serum T levels in 10 and 50-BS groups were decreased at day 46, and in 250 -BS and TP groups were significantly increased at day 31, and the levels were returned to the pre-treatment levels in all groups, thereafter. However, the T levels were increased again at day 47-62 in 250 - BS group. When compared to the DW group, serum T levels at day 46 in 10 and 50-BS groups were lower than the DW group, whereas the level was higher than the DW group at day 31 and 47-62 in 250-BS group. There were no significant difference of T levels throughout study period between TP and DW group (Figure 22 and Table 7).

Hormone levels	Treatment	Day of blood collection						
		1	16	31	46	47	61	62
Testosterone (pg/ml)	DW	283.930±61.56	371.646±62.51	297.274±47.27	349.278±94.10	239.478±70.95	353.828±109.66	493.794±134.60
	10-BS	296.444±49.81	328.317±74.16	382.940±131.96	185.586±58.79^{a}	292.058±20.78	262.302±80.18	579.668±133.20^{**,b}
	50-BS	292.818±69.29	408.487±131.61	373.679±113.93	102.412±35.39^{a}	245.008±165.74	396.308±116.78	284.416±77.42
	250-BS	291.928±39.45	547.417±122.46	587.370±113.63^{*,a}	399.541±110.58	672.422±219.91^{**,a2,b2}	619.594±137.24^{**,a,b2}	783.940±145.27^{**,a,b2}
	TP	297.372±48.84	455.275±37.67	482.884±29.26*	452.426±37.98	285.802±26.98	146.490±23.84^{a}	139.600±35.45^{a}
FSH (ng/ml)	DW	5.437±0.48	5.801±0.45	6.052±0.52	5.099±0.30	4.989±0.67	5.214±0.42	5.190±0.42
	10-BS	4.908±0.24	5.256±0.44	5.867±0.52^{b}	5.044±0.34^{b}	4.957±0.59^{b}	5.064±0.43	4.445±0.50
	50-BS	4.724±0.22	4.722±0.35	5.308±0.28^{b}	4.387±0.42^{b}	3.657±0.71^{b}	4.675±0.50	4.471±0.24
	250-BS	5.091±0.53	3.987±0.30	5.001±0.51^{b}	4.510±0.59^{b}	5.608±0.33^{b2}	3.212±0.99	4.072±0.33
	TP	4.798±0.49	4.305±0.52	1.905±0.11^{*,a2}	1.543±0.11^{*,a2}	1.480±0.12^{*,a2}	3.406±1.24	3.410±1.19
LH (ng/ml)	DW	0.643±0.240	0.651±0.120	1.028±0.210	0.442±0.150	0.415±0.170	0.684±0.140	0.916±0.280
	10-BS	0.381±0.050	0.971±0.310	1.436±0.220^{b2}	0.619±0.150^{b}	0.580±0.270^{b}	0.742±0.170	1.262±0.290^{b2}
	50-BS	0.481±0.120	0.912±0.230	1.162±0.200^{b}	0.457±0.140	0.365±0.190	1.175±0.360^{b2}	1.230±0.510^{b2}
	250-BS	0.493±0.090	0.891±0.090	0.930±0.090^{b}	0.971±0.220^{a,b2}	1.341±0.450^{a,b2}	0.951±0.430^{b2}	1.599±0.350^{b2}
	TP	0.544±0.120	0.855±0.170	0.047±0.008^{**,a}	0.041±0.005^{**,a}	0.039±0.007^{**,a}	0.098±0.020^{**,a}	0.163±0.040^{**,a}

Table 7. serum T, FSH and LH levels in normal male rats treated with distilled water, *B. superba* and testosterone propionate

*, **=P<0.05 and P<0.01 compared to the pre-treatment levels (day 1)

a ,a_2= P<0.05 and P<0.01 compared to DW group

b, b_2= P<0.05 and P<0.01 compared to TP group

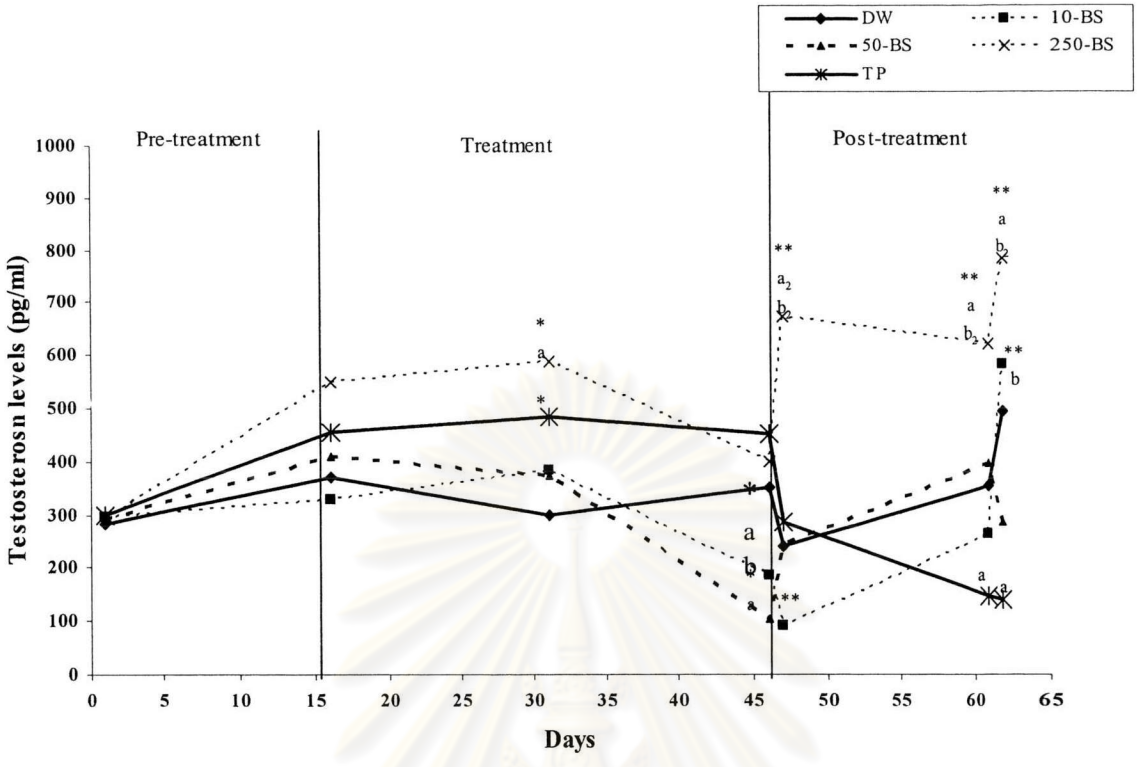


Figure 22. Serum T levels in normal male rats treated with distilled water, *B. superba*, and testosterone propionate.

*,** = P<0.05 and P<0.01 compared to the pre-treatment levels (day 1)

a, a_2 = P<0.05 and P<0.01 compared to DW group

b, b_2 = P<0.05 and P<0.01 compared to TP group

Serum FSH levels

When compared to the pre-treatment levels (day 1), serum FSH levels in DW and BS groups were not changed throughout the study period, whereas the levels were highly significantly decreased (p<0.01) since day 31 until the first day of post-treatment period (day 47) in TP group. FSH levels were returned to the pre- treatment levels after the TP cessation for 2 weeks. The decrease of serum FSH levels between day 31 - 47 in TP group was also highly significant difference from the BS groups. The BS groups did not show

any significant deference of serum FSH levels from the DW group in all 3 treatment periods (Figure 23 and Table 7).

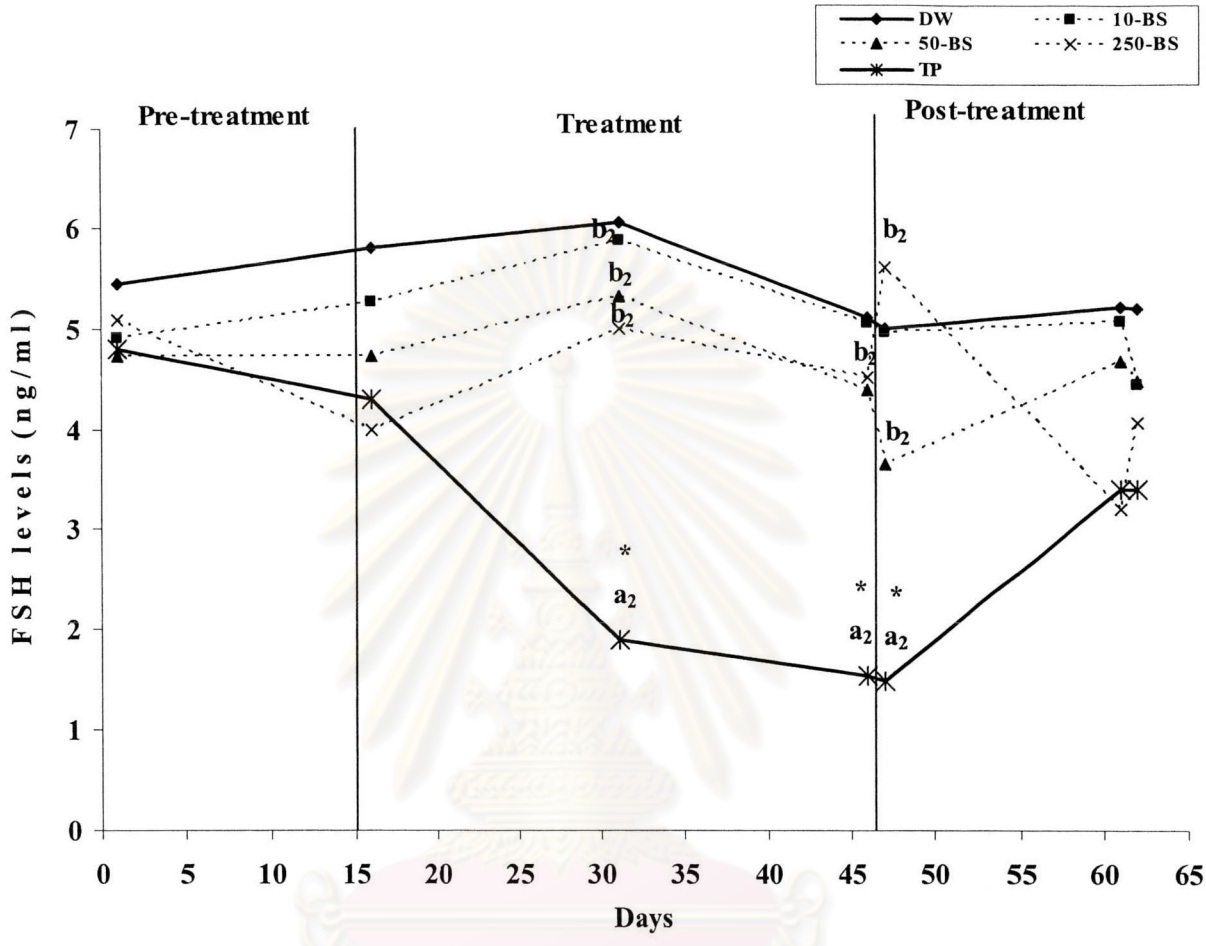


Figure 23. Serum FSH levels in normal male rats treated with distilled water, *B. superba*, and testosterone propionate.

* = $P<0.05$ compared to the pre-treatment levels (day 1)

a_2 = $P<0.01$ compared to DW group

b_2 = $P<0.01$ compared to TP group

Serum LH levels

When compared to the pre-treatment levels (day 1), serum LH levels in DW and BS groups were not changed throughout the study period, whereas the levels were highly significantly decreased ($p<0.01$) since day 31 until the last day of post-treatment period (day 62) in TP group. Moreover, the decrease of serum LH levels between day 31 - 62 in TP group was also highly significant difference from the DW and BS groups. The BW groups did not show any significant difference of serum LH levels from the DW group in all 3 treatment periods (Figure 24 and Table 7).

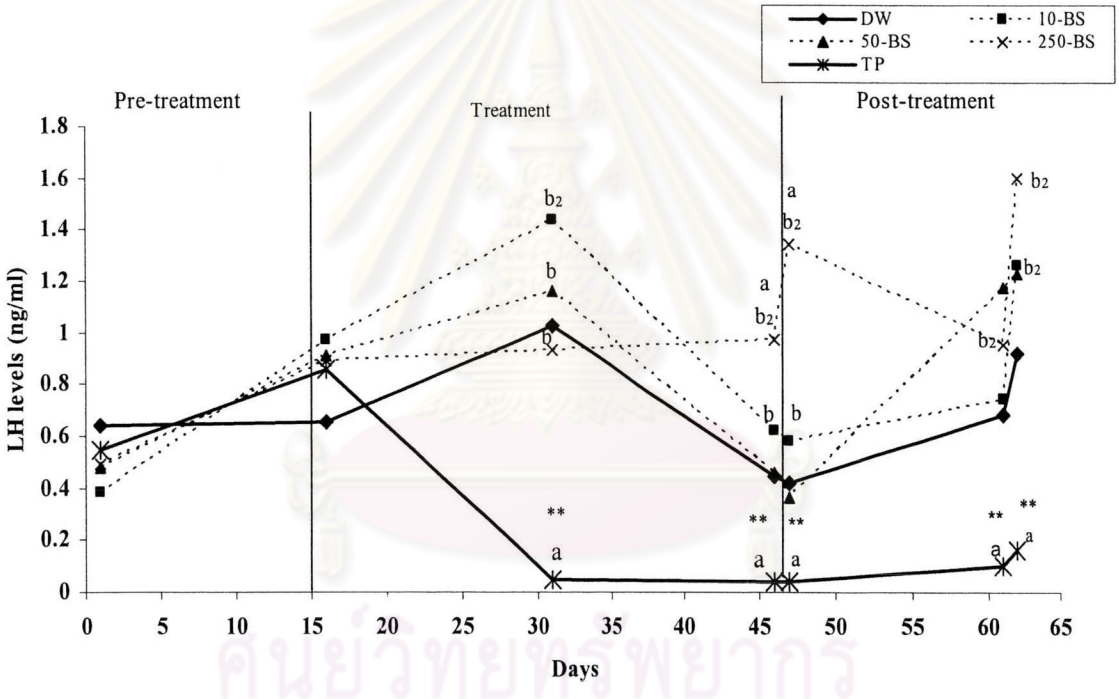


Figure 24. Serum LH levels in normal male rats treated with distilled water, *B. superba*, and testosterone propionate.

** = $P< 0.01$ compared to the pre-treatment levels (day 1)

a = $P<0.05$ compared to DW group

b, b_2 = $P<0.05$ and $P< 0.01$ compared to TP group

The body weights

When compared to the pre-treatment period (day 1), body weights in rats were significantly increased after 7 days of DW, BS and TP administration and reached the plateau at day 30 of treatment period.

When compared to the DW and TP groups, body weights is rat treated with BS did not difference from the DW and TP groups throughout the study period (Figure 25).

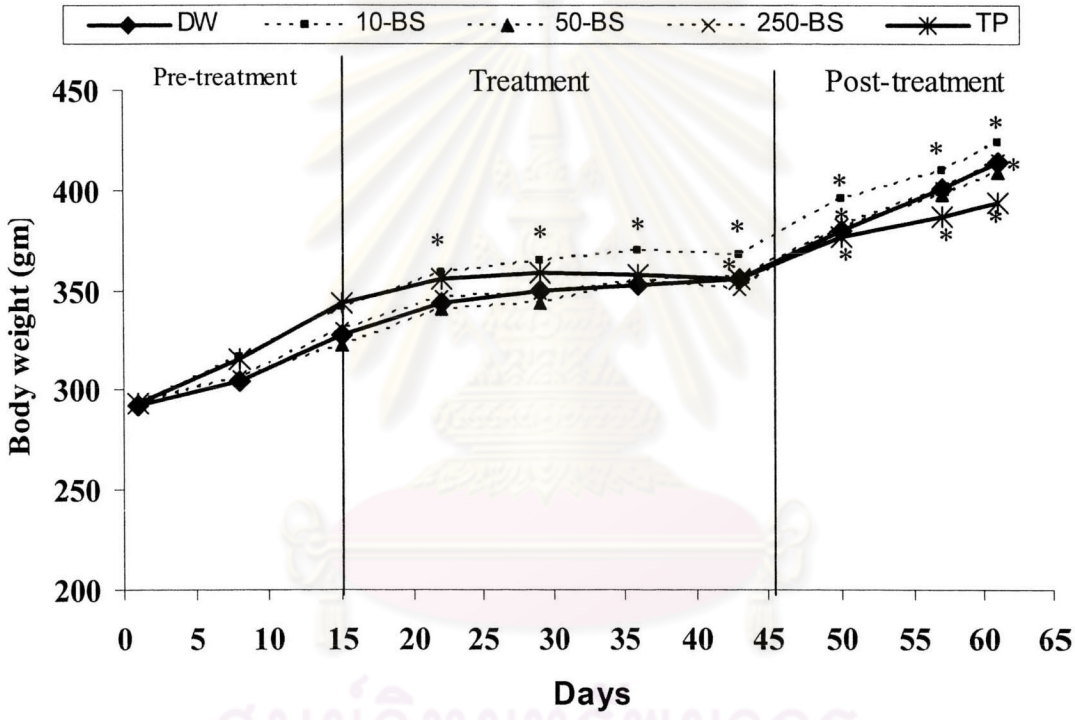


Figure 25. Body weights in normal male rats treated with distilled water, *B. superba*, and testosterone propionate.

* = P<0.05 compared to the pre-treatment levels (day 1)

The weight and absolute weight of reproductive organs

Weight and absolute weight of testis

When compared the weight and the absolute weight of testis between the end of treatment and the end of post-treatment periods, there were no difference in DW and BS groups, but the weights at the end of post-treatment period were significantly lower than the end of treatment period in TP group. When compared to the DW group, the weight and the absolute weight of testis in TP group were lower than the DW group in both period, and also lower than all BS groups (Table 8).

	Treatment	testis weight		absolute testis weight (organ weight/body weight)	
		end of treatment (gm)	end of post-treatment (gm)	end of treatment ($x10^{-3}$)	end of post-treatment ($x10^{-3}$)
Testis	DW	3.48±0.127	3.628±0.187	11.027±0.241	8.998±0.64
	10-BS	3.612 ± 0.102^{b2}	3.698 ± 0.172^{b2}	11.449 ± 0.424^{b2}	8.995 ± 0.393^{b2}
	50-BS	3.65 ± 0.102^{b2}	3.544 ± 0.166^{b2}	11.830 ± 0.204^{b2}	8.836 ± 0.528^{b2}
	250-BS	3.68 ± 0.291^{b2}	3.682 ± 0.081^{b2}	11.276 ± 0.906^{b2}	8.370 ± 0.437^{b2}
	TP	1.812 ± 0.047^{a2}	$1.484\pm0.067^{a2,**}$	5.213 ± 0.123^{a2}	$3.778\pm0.136^{a2,**}$

Table 8. The weights and the absolute weights of testis in normal male rats treated with distilled water, *B. superba* and testosterone propionate.

**= $P<0.01$compared to the end of treatment periods

a_2 = $P<0.01$ compared to DW group

b_2 = $P<0.01$ compared to TP group

Weight and absolute weight of epididymis

When compared the weight of epididymis between the end of treatment and the end of post-treatment periods, no differences were found in DW and BS groups, but the absolute epididymis weights at the end of post-treatment period were lower than at the end of treatment period in all BS groups. The epididymis weight and absolute weight at the end of treatment period were also significantly lower than at the end of post-treatment period in TP groups. The weight and the absolute weight of epididymis of both periods in TP group were significantly higher than the DW and also all BS groups (Table 9).

	Treatment	epididymis weight		absolute epididymis weight (organ weight/body weight)	
		end of treatment (gm)	end of post-treatment (gm)	end of treatment ($x10^{-3}$)	end of post-treatment ($x10^{-3}$)
Epididymis	DW	1.122±0.06	1.368±0.049	3.550±0.157	3.401±0.237
	10-BS	$1.136±0.064^{b2}$	$1.198±0.053^{a,b2}$	$3.607±0.248^{b2}$	$2.917±0.141^{*,b2}$
	50-BS	$1.114±0.029^{b2}$	$1.066±0.068^{a,b2}$	$3.618±0.161^{b2}$	$2.649±0.178^{**,b2}$
	250-BS	$1.234±0.038^{b2}$	$1.206±0.523^{a,b2}$	$3.773±0.069^{b2}$	$2.732±0.133^{**,b2}$
	TP	$3.682±0.025^{a2}$	$2.744±0.016^{**,a2}$	$7.965±0.083^{a2}$	$4.387±0.043^{**,a2}$

Table 9. The weights and the absolute weights of epididymis in normal male rats treated with distilled water, *B. superba* and testosterone propionate.

*, **= $P<0.05$ and $P<0.01$ compared to the end of treatment period

a,a_2 = $P<0.05$ and $P<0.01$ compared to DW group

b_2 = $P<0.01$ compared to TP group

Weight and absolute weight of seminal vesicle

When compared the weight and the absolute weight of seminal vesicle between the end of treatment and the end of post-treatment periods, no differences in DW, 10 and 50 – BS groups were found. But the values at the end of post-treatment period was significantly lower than the end of treatment period in TP and 250-BS groups. When compared to the DW group, the absolute weights in TP group and the weights in TP and BS groups was significantly higher than the DW group at the end of treatment period. The increase of the weight of seminal vesicle was returned to the DW group values after 2 weeks of 250-BS cessation. The increase of the weight and the absolute weight of seminal vesicle in TP group were also significantly higher than that of all BS groups in both periods (Table 10).

	Treatment	seminal vesicle weight		absolute seminal vesicle weight (organ weight/body weight)	
		end of treatment (gm)	end of post-treatment (gm)	end of treatment ($x10^{-3}$)	end of post-treatment ($x10^{-3}$)
Seminal vesicle	DW	0.851±0.065	1.043±0.48	2.701±0.21	2.830±0.166
	10-BS	0.868±0.152^{b2}	1.163±0.173^{b2}	2.39±0.189^{b2}	3.128±0.494^{b2}
	50-BS	0.806±0.149^{b2}	1.202±0.182^{b2}	2.562±0.404^{b2}	3.070±0.54^{b2}
	250-BS	1.452±0.143^{a2,b2}	1.056±0.082^{b2,*}	4.430±0.39^{b2}	2.399±0.213^{b2,**}
	TP	3.433±0.191^{a2}	1.906±0.124^{a2,**}	9.850±0.405^{a2}	4.859±0.311^{a2,**}

Table 10. The weight and the absolute weight of seminal vesicle in normal male rats treated with distilled water, *B. superba* and testosterone propionate.

*, **= $P<0.05$ and $P<0.01$ compared to the end of treatment period

a_2 = $P<0.01$ compared to DW group

b_2 = $P<0.01$ compared to TP group

Histological study of reproductive organs

Histology of testis

The histology of rat testis treated with DW, BS and TP at the end of treatment and at the end of post-treatment periods was studied. The number of seminiferous tubule that showed the decrease of spermatozoa was counted in there serial sections from the largest cross-section through the center of the testis and averaged. The result showed the numerous of spermatogenic cell in various stages, primary spermatocytes, secondary spermatocytes, spermatids and spermatozoa in DW and BS groups. In contrast, testes of rats treated with TP showed a thin layer of spermatogenic linage and the decrease of spermatozoa when compared to the DW and BS groups (Figure 26 and Table 11).

The histology of rat testis treated with DW and BS groups at the end of post-treatment period also showed the numerous of spermatogenic cell in various stages, primary spermatocytes, secondary spermatocytes, spermatids and spermatozoa. However, those spermatogenic cell types were decreased in the testes of rat treated with TP (Figure 27 -28 and Table 11).

There were no differences between the end of treatment and the end of post-treatment period in all treatment groups.

Treatment	% of seminiferous tubule that showed the decrease of spermatozoa per section	
	end of treatment (%)	end of post-treatment (%)
DW	24.149±0.691	23.227±0.894
10-BS	25.667±0.476^{b2}	24.276±1.053
50-BS	23.955±0.804^{b2}	23.772±1.959
250-BS	25.043±1.452^{b2}	24.126±1.195
TP	47.859±2.752^{a2}	28.006±2.037^{**,a2}

Table 11. The percentage of seminiferous tubule that showed the decrease of spermatozoa per section of rat treated with distilled water, *B. superba* and testosterone propionate at the end of treatment and the end of post-treatment

** = P<0.01 compared to the end of treatment period

a_2 = P<0.01 compared to DW group

b_2 = P<0.01 compared to TP group

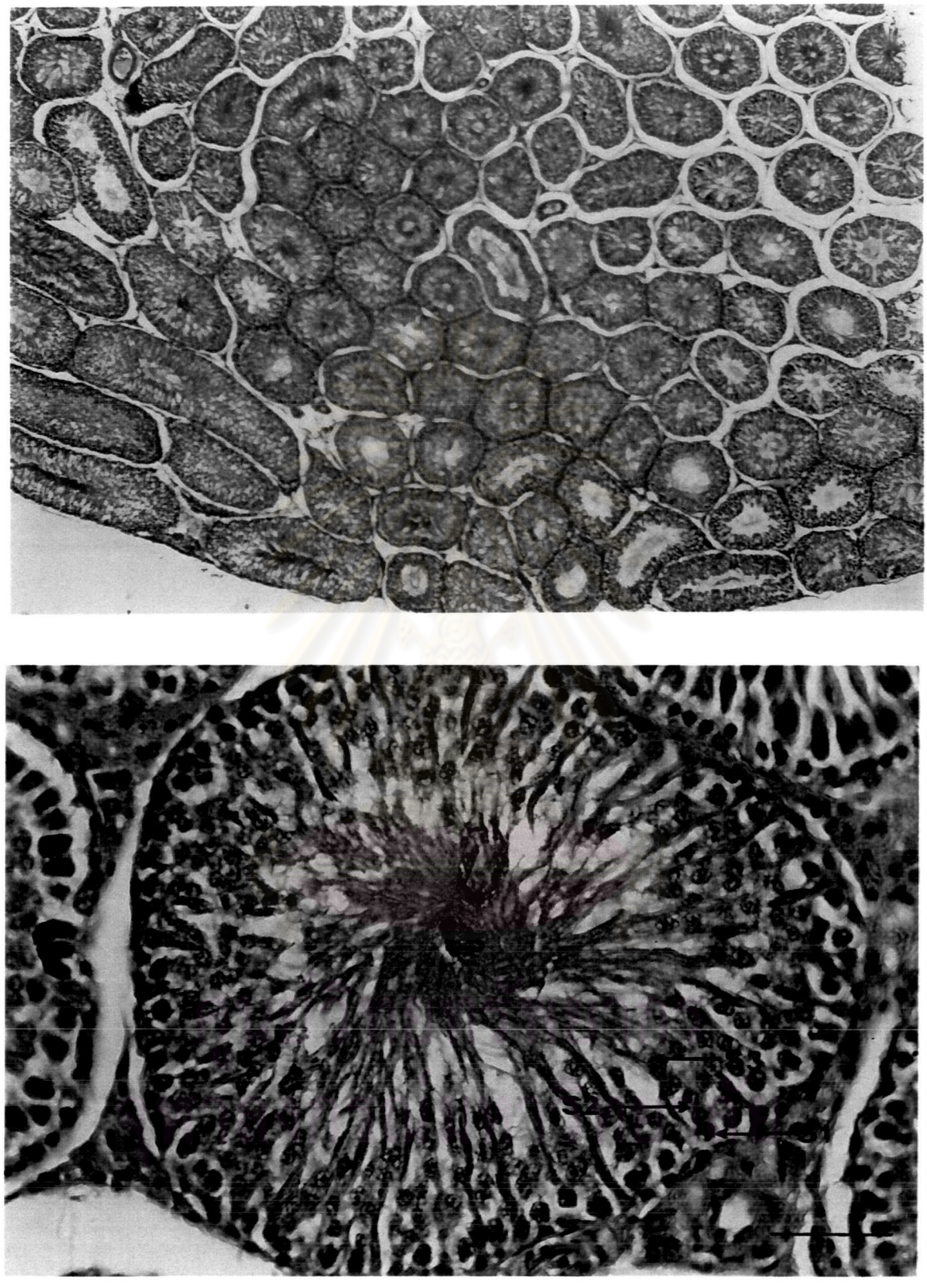
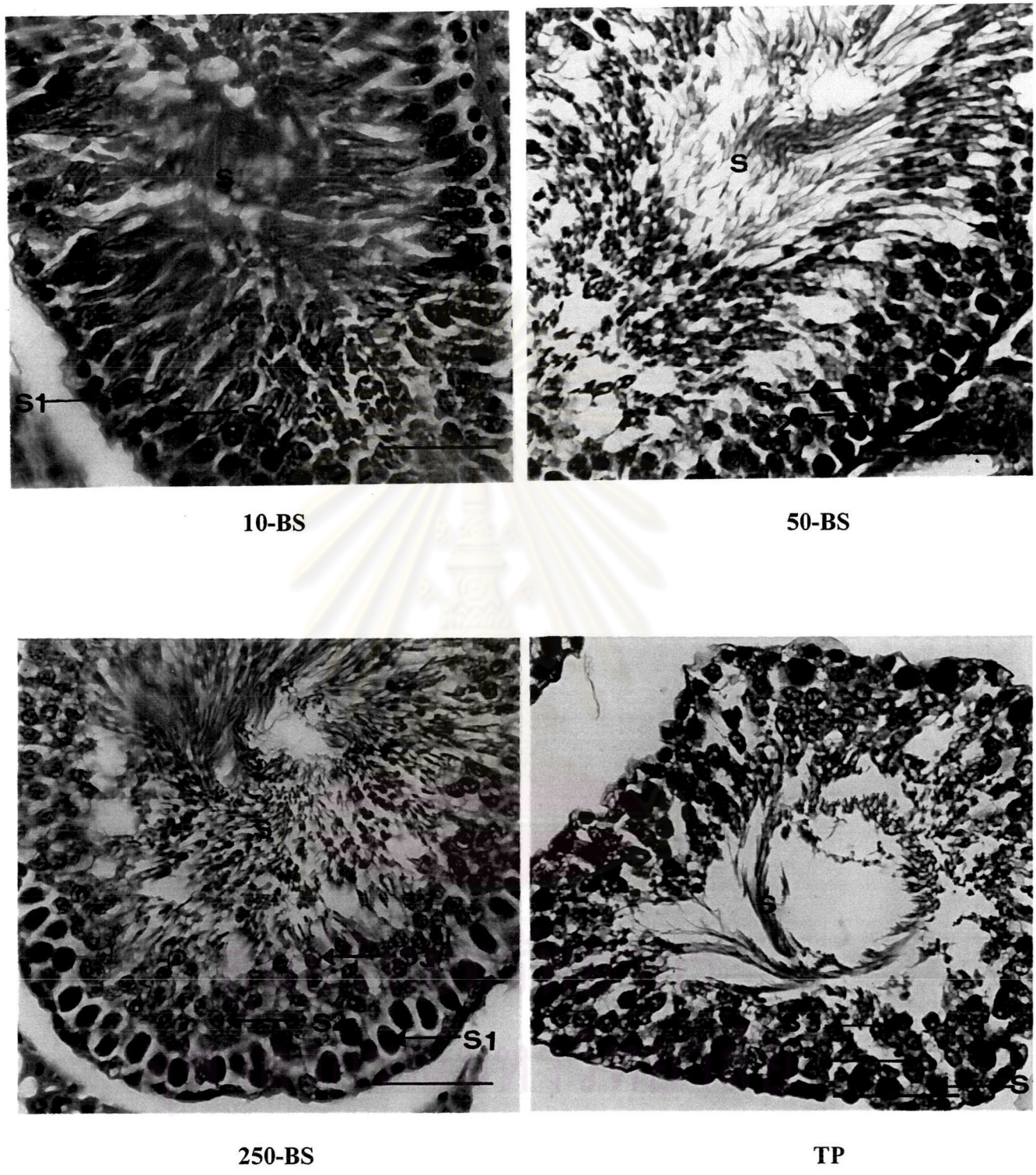


Figure 26. Testicular morphology in DW group of normal male rats at the end of treatment period. S1=Primary spermatocyte, S2=Secondary spermatocyte, S3=Spermatid and S=Spermatozoa. H&E stain. (Scale bars=50 μm)



10-BS **50-BS**

250-BS **TP**

Figure 27. Testicular morphology in normal male rats treated with 10-BS, 50-BS, 250-BS and TP at the end of treatment period. S1=Primary spermatocyte, S2=Secondary spermatocyte, S3=Spermatid and S=Spermatozoa. H&E stain. (Scale bars=50 μm)

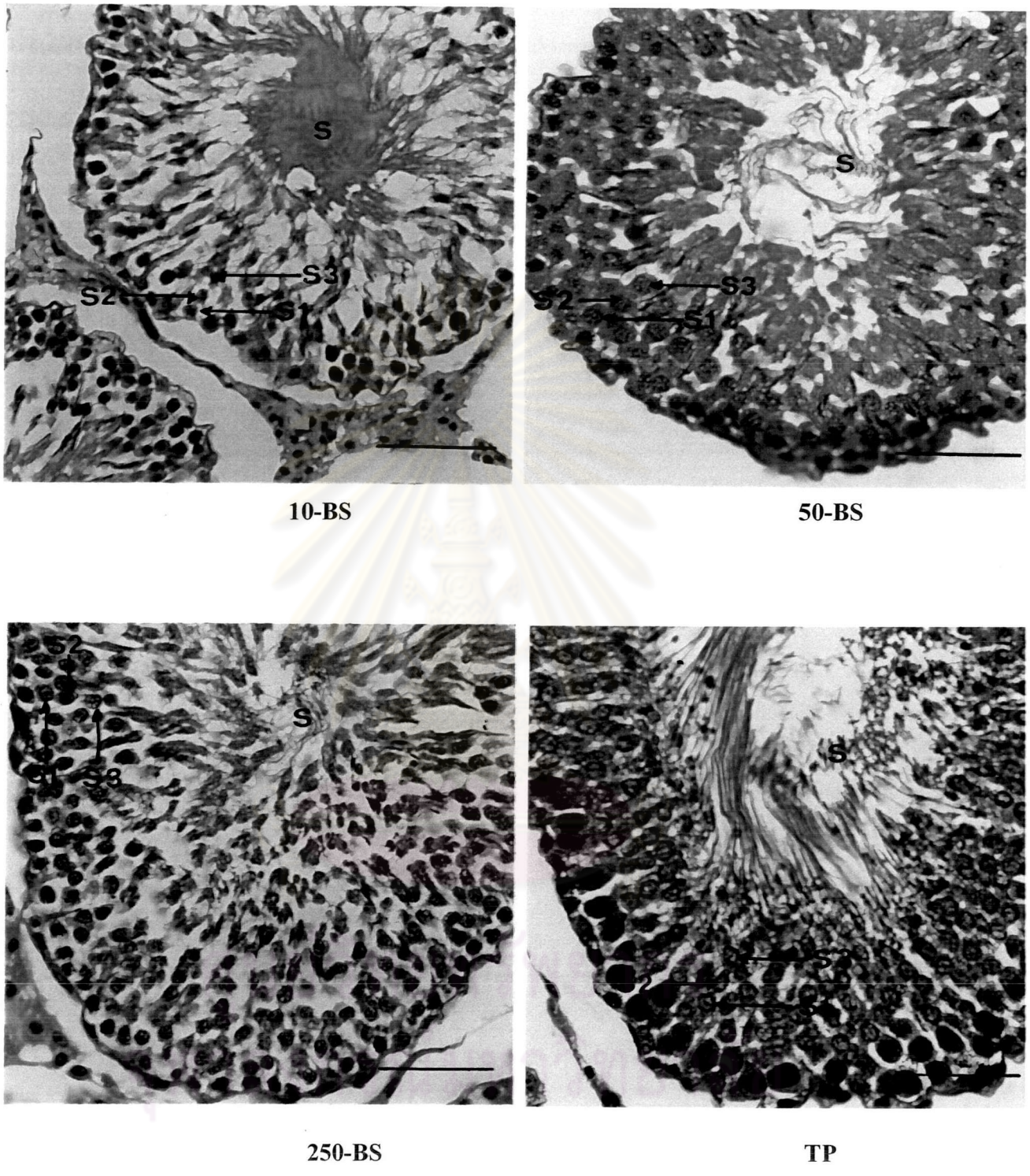


Figure 28. Testicular morphology in normal male rats treated with 10-BS, 50-BS, 250-BS and TP at the end of post-treatment period. S1=Primary spermatocyte, S2=Secondary spermatocyte, S3=Spermatid and S=Spermatozoa. H&E stain. (Scale bars=50 μm)

Histology of epididymis

The histological study of epididymis in rats treated with DW and BS groups at the end of treatment period showed the columnar cell and the numerous of spermatozoa. In contrast, epididymis of rats treated with TP showed a thick layer of epithelium, but the numbers of spermatozoa were decreased (Figure 29).

The histological study of epididymis in rats treated with DW and BS groups at the end of post-treatment period showed the columnar cell and the numerous of spermatozoa. In contrast, epididymis of rats treated with TP showed a thinner layer of epithelium and the less number of spermatozoa when compared to the end of treatment period. However, no differences between that two periods were found in DW and BS groups (Figure 30-31).

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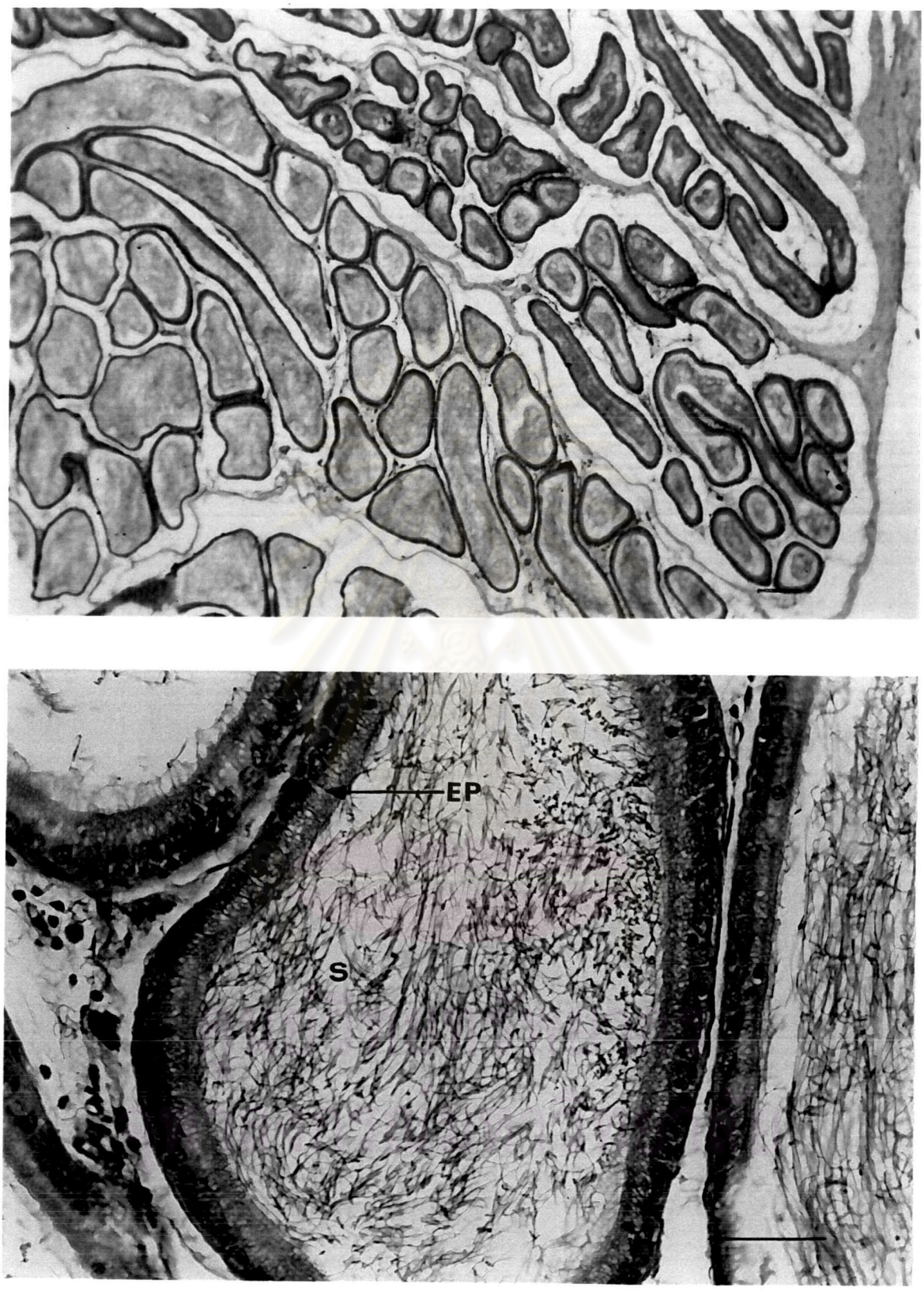


Figure 29. Epididymis morphology in DW group of normal male rats at the end of treatment period. EP=Epithelium, S=Spermatozoa. H&E stain. (Scale bars=50 μm)

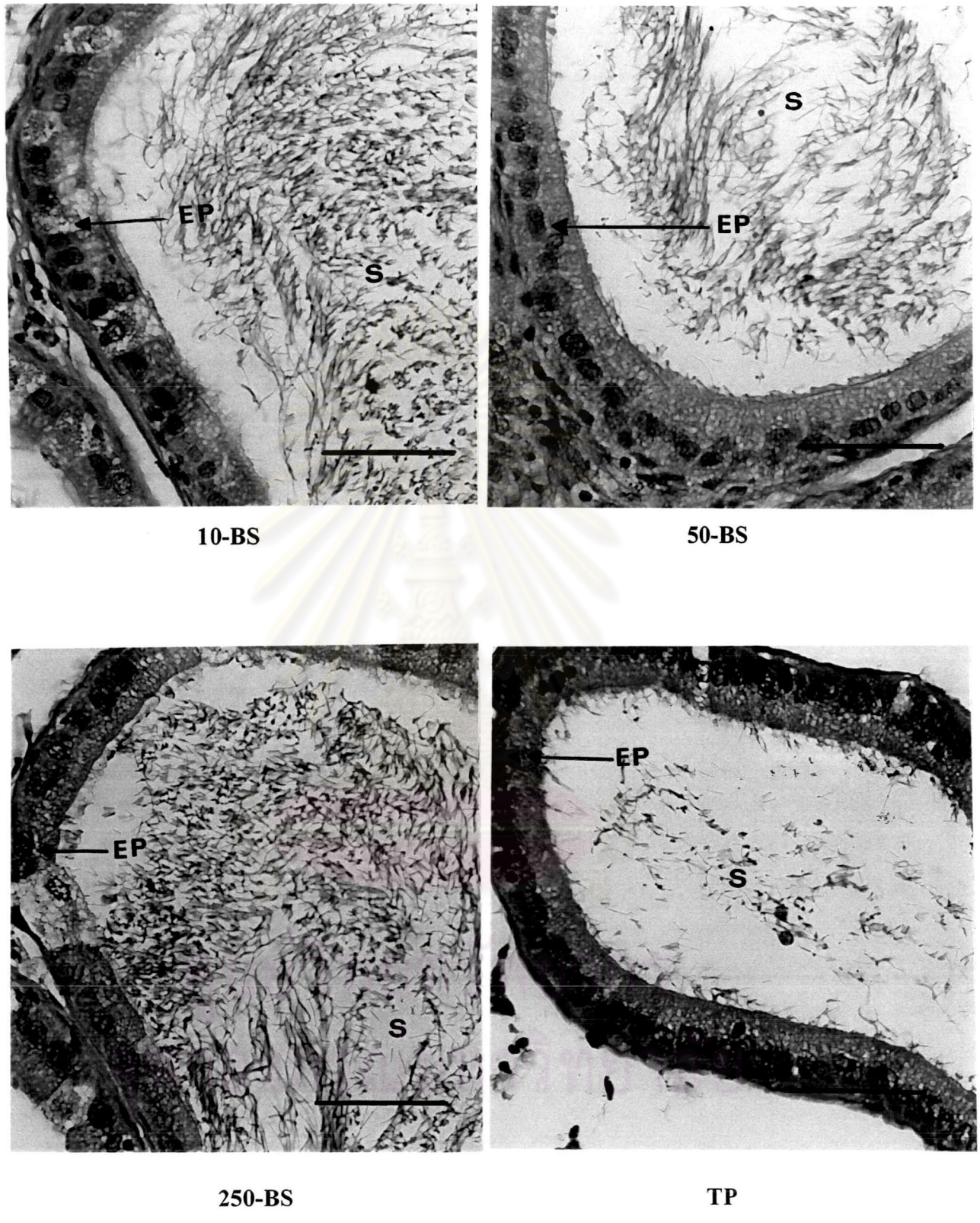


Figure 30. Epididymis morphology in normal male rats treated with 10-BS, 50-BS, 250-BS and TP at the end of treatment period. EP=Epithelium, S=Spermatozoa. H&E stain. (Scale bars=50 μm)

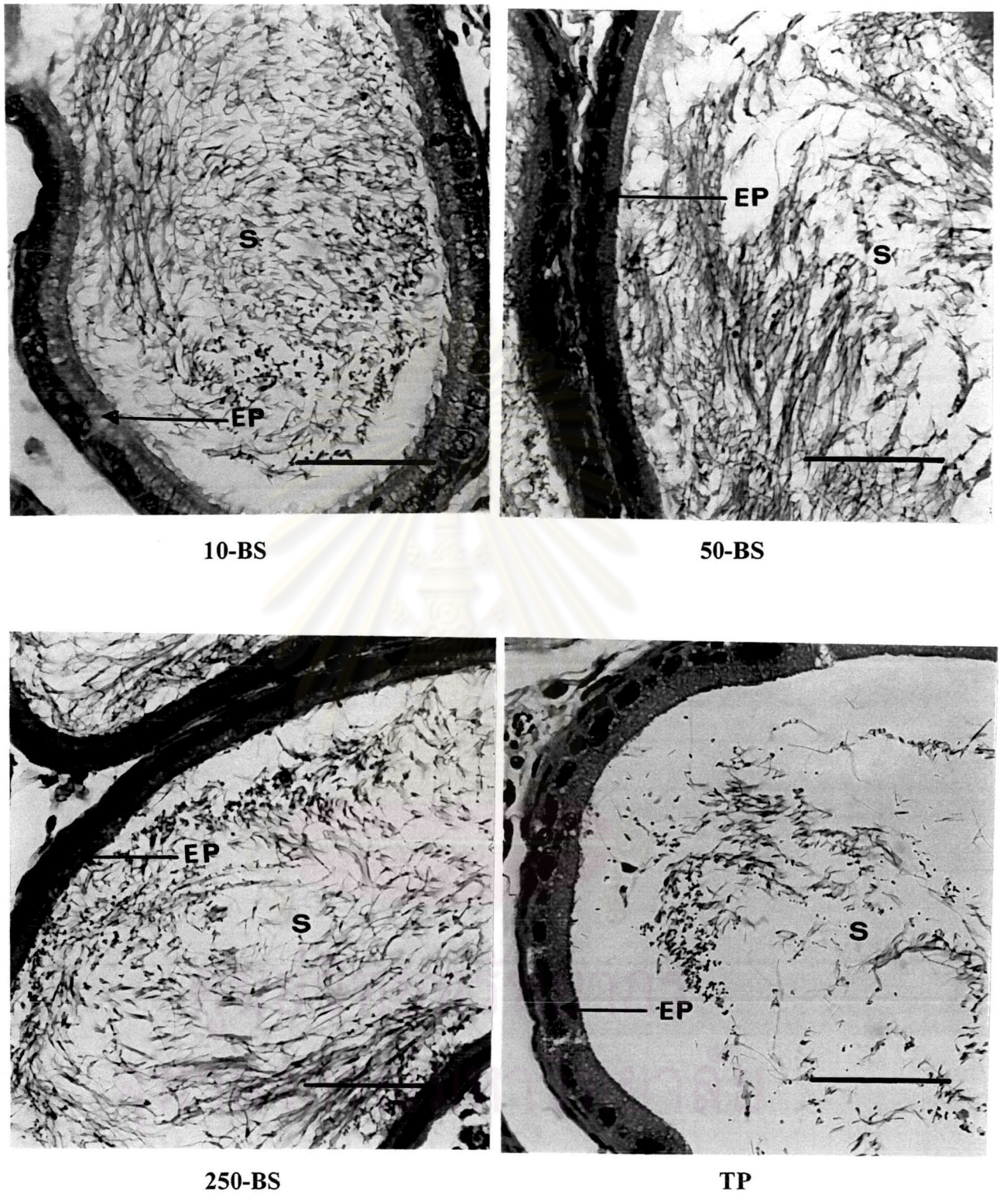


Figure 31. Epididymis morphology in normal male rats treated with 10-BS, 50-BS, 250-BS and TP at the end of post-treatment period. EP=Epithelium, S=Spermatozoa. H&E stain. (Scale bars=50 μm)

Histology of seminal vesicle

The histology of seminal vesicle in normal male rat treated with DW and BS at the end of treatment period showed the highly folded tubular glands and muscular layers. There were a secretion material in lumen of seminal vesicle. Whereas, in the TP group, the folded tubular glands and secretion material were higher than the DW and BS group (Figure 32).

The histological study of seminal vesicle of normal male rat treated with DW and BS groups at the end of post treatment period showed a papilla folding of tubular glands. A whitish-yellow viscous material was secreted into the lumen of seminal vesicle. A papilla folding of tubular glands in TP group were higher than the DW and BS groups (Figure 33-34).

There were no differences between the end of treatment and the end of post-treatment in DW and BS groups, except the TP group, the folded tubular glands and secretion material at the end of post-treatment period were lower than at the end of treatment period.

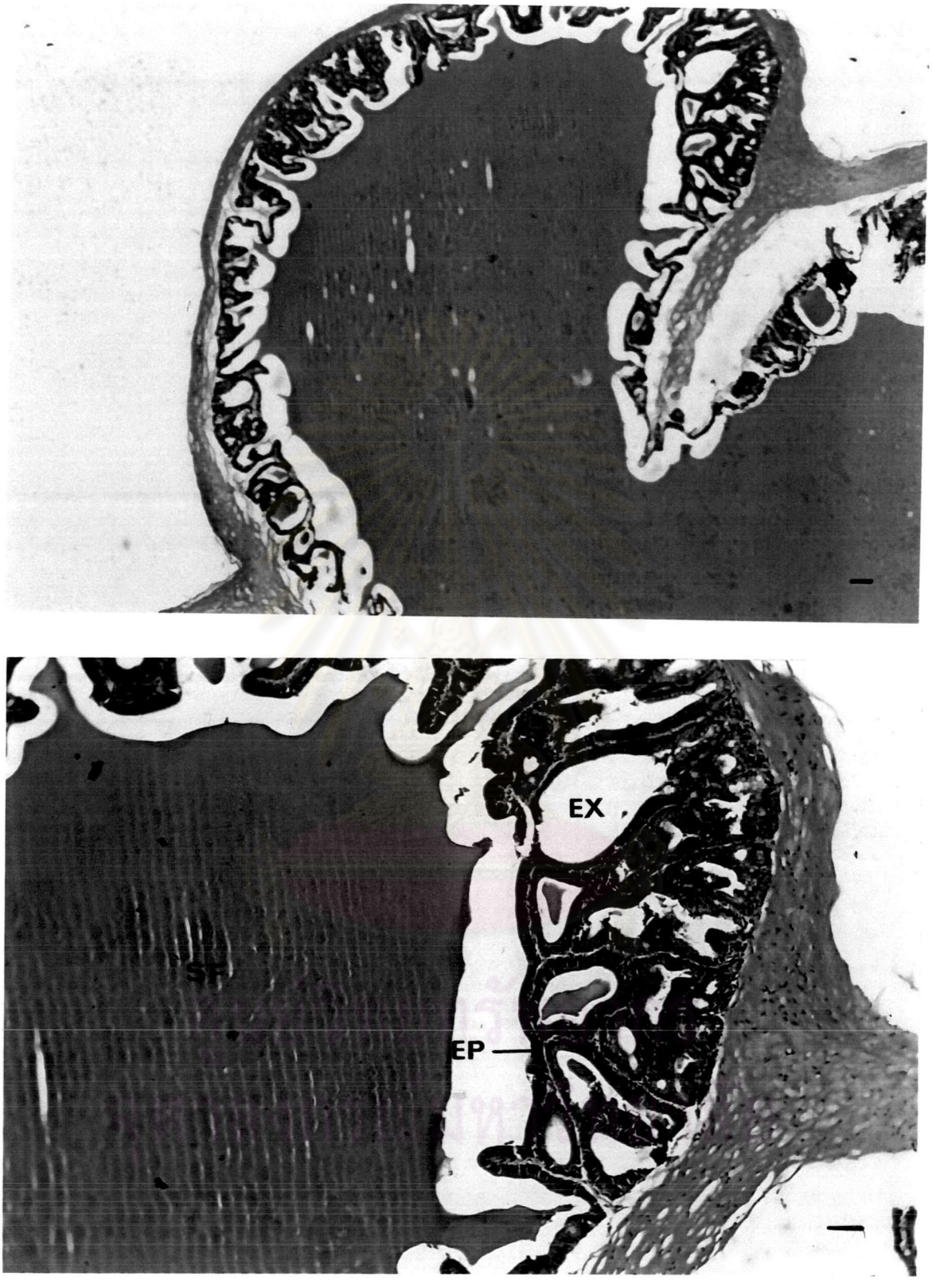


Figure 32. Seminal vesicle morphology in DW group in normal male rats at the end of treatment period. EX=Extensive folding, EP=Epithelium cell, SF= Seminal fluid. H&E stain. (Scale bars=50 μm)

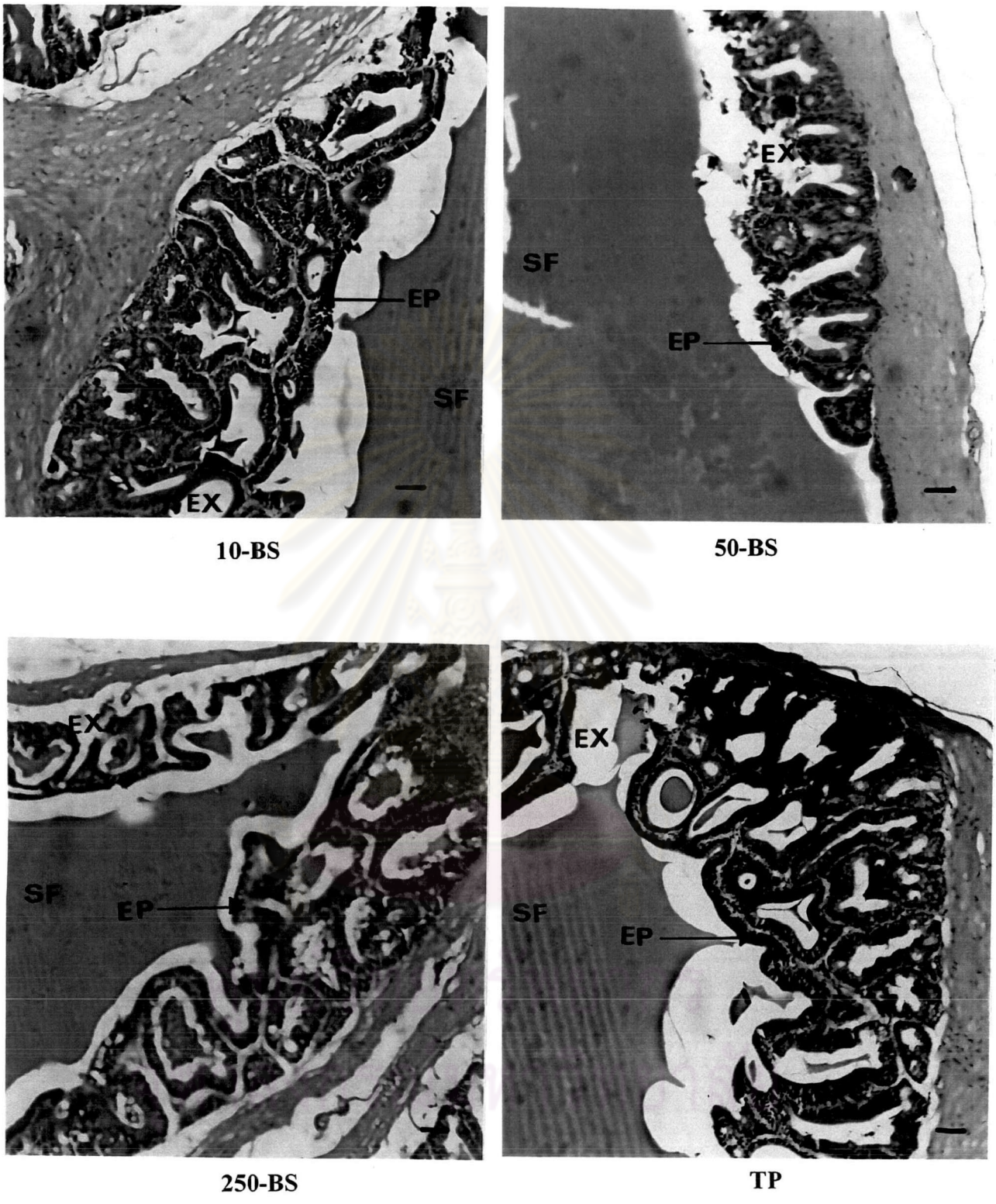
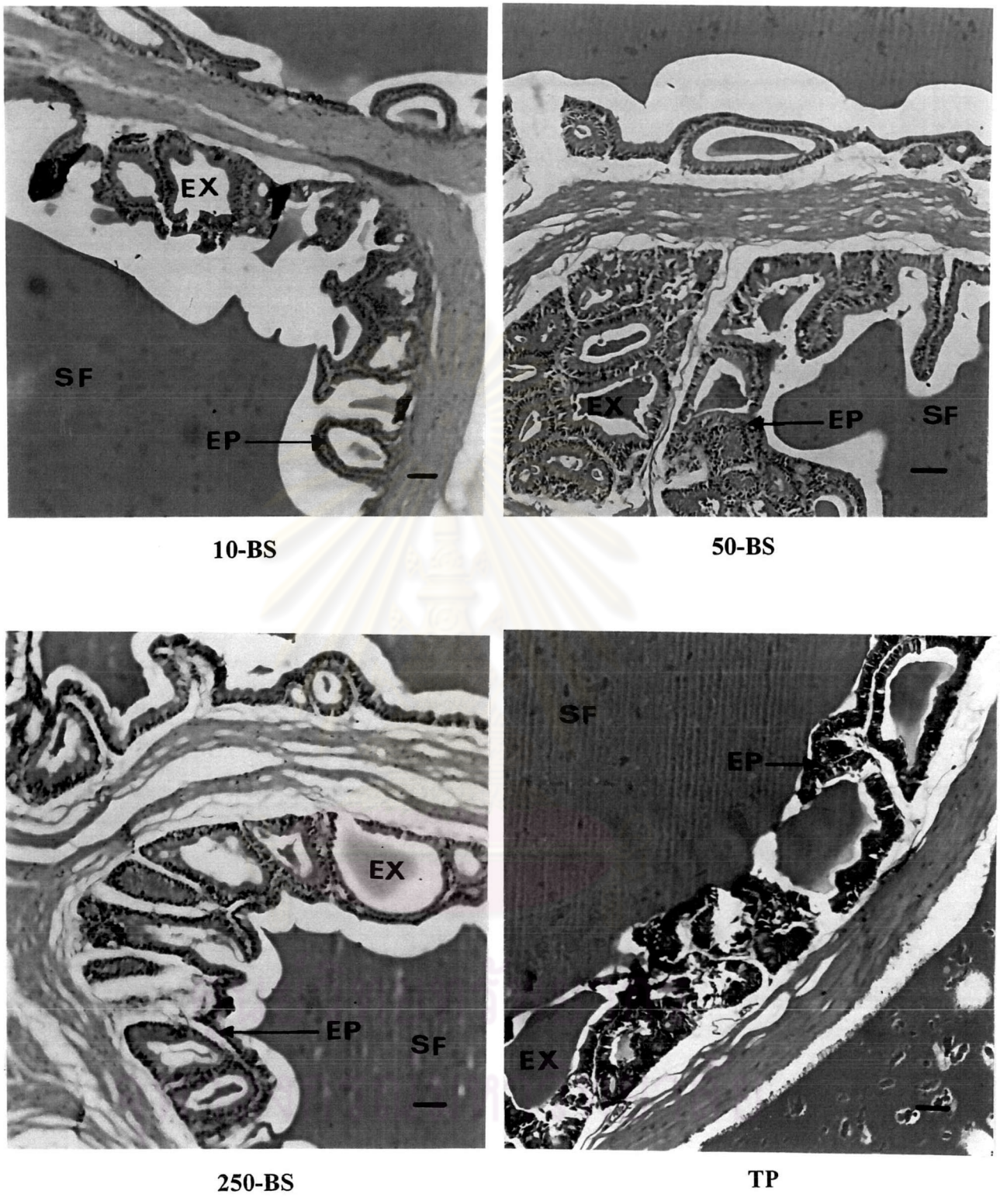


Figure 33. Seminal vesicle morphology in normal male rats treated with 10-BS, 50-BS, 250-BS and TP at the end of treatment period. EX=Extensive folding, EP=Epithelium cell, SF= Seminal fluid. H&E stain. (Scale bars=50 μm)



10-BS 50-BS

250-BS TP

Figure 34. Seminal vesicle morphology in normal male rats treated with 10-BS, 50-BS, 250-BS and TP at the end of post-treatment period. EX=Extensive folding, EP=Epithelium cell, SF= Seminal fluid. H&E stain. (Scale bars=50 μm)

Experiment 4: The effect of *B. superba* on sex hormone levels and reproductive organs in orchidectomized rats.

Serum T levels

After orchidectomy for 14 days, serum T levels were significantly decreased in all 5 groups (286.114±56.93 pg/ml at day -14 and 17.72±2.07 pg/ml at day 1, respectively). When compared to the pre-treatment levels (day 1) serum T levels in DW and BS groups were not change throughout the study period, whereas the serum T levels in TP group were highly significantly increased ($p<0.01$) since day 16 until the first day of post treatment period (day 47) and decreased toward the ODX levels, thereafter. Moreover, the increase of serum T levels at day 16 to day 47 in TP group was also highly significant difference from the DW and BS groups. The BS group did not show any significant difference of serum T levels from the DW group in all 3 treatment periods (Figure 35 and Table 12).

Hormone levels	Treatment	Day of blood collection							
		-14	1	16	31	46	47	61	62
Testosterone (pg/ml)	DW	302.238±56.93	25.376±2.07	23.323±1.89	25.793±1.56	26.792±1.95	28.120±1.81	27.608±2.24	23.538±3.30
	10-BS	313.802±46.52	23.409±3.22	31.926±2.85^{b2}	27.858±2.62^{b2}	31.787±3.92^{b2}	29.930±2.45^{b2}	22.686±4.55^{b}	24.202±3.82^{b}
	50-BS	273.978±44.60	18.867±2.42	23.066±4.12^{b2}	21.405±2.21^{b2}	29.778±1.99^{b2}	20.376±4.69^{b2}	20.228±4.59^{b}	20.482±2.06^{b}
	250-BS	273.148±38.67	20.696±0.89	28.816±1.30^{b2}	26.212±2.91^{b2}	23.282±1.98^{b2}	28.390±1.81^{b2}	20.968±2.53^{b}	25.254±1.79^{b}
	TP	267.397±27.86	10.230±1.22	945.385±38.39^{**,a2}	1078.892±28.84^{**,a2}	994.263±13.60^{**,a2}	1019.382±21.86^{**,a2}	169.600±76.60^{**,a2}	182.234±79.51^{**,a2}
FSH (ng/ml)	DW	5.220±0.33	30.439±2.88	40.815±2.49	63.699±4.39*	74.884±6.45*	85.481±14.80*	69.237±2.84*	69.115±13.17*
	10-BS	4.337±0.24	24.336±1.94	38.608±4.39	51.077±8.44^{b2}	52.287±4.58^{b2}	69.313±13.90^{b2}	51.339±3.95*	46.156±8.04*
	50-BS	6.001±1.77	26.236±1.62	37.097±2.28	47.343±4.25^{b2}	45.427±5.31^{b2}	54.711±7.51^{b2}	44.815±8.74*	38.614±9.21*
	250-BS	5.583±0.26	24.076±2.36	39.049±2.04	49.353±2.76^{b2}	54.001±3.59^{b2}	54.548±5.20^{b2}	56.022±5.41*	47.059±3.73*
	TP	5.272±0.41	27.004±1.49	35.345±2.27	11.917±0.73^{**,a2}	7.780±0.65^{**,a2}	9.155±1.00^{**,a2}	38.746±11.45*	36.224±10.46*
LH (ng/ml)	DW	0.514±0.12	40.843±7.64	66.473±12.96	146.909±46.06**	135.862±78.91**	125.745±39.31**	151.774±20.27**	133.474±27.88**
	10-BS	0.516±0.13	25.463±5.13	41.215±9.77	77.143±24.23^{*,b}	153.297±89.04^{**,b2}	157.920±58.24^{**,b2}	112.332±15.22^{**,b2}	101.373±79.75^{**,b2}
	50-BS	0.801±0.05	14.285±0.44	24.129±1.30	23.993±0.91^{a2,b}	33.155±3.81^{a2,b}	43.864±22.00^{a2,b}	26.912±4.75^{a2}	31.135±4.39^{a2}
	250-BS	0.499±0.16	15.178±2.21	21.732±2.69	26.269±1.83^{a2,b}	25.125±3.49^{a2,b}	29.728±7.94^{a2,b}	34.493±6.80^{a2}	23.684±2.55^{a2}
	TP	0.969±0.33	11.129±1.02	17.366±0.84	0^{*,a2}	0^{*,a2}	0^{*,a2}	8.688±3.82^{a2}	9.9082±3.88^{a2}

Table 12. serum T, FSH and LH levels in orchidectomized rats treated with distilled water, *B. superba* and testosterone propionate

*, **=P<0.05 and P<0.01 compared to the pre-treatment levels (day 1)

a_2= P<0.01 compared to DW group

b, b_2= P<0.05 and P<0.01 compared to TP group

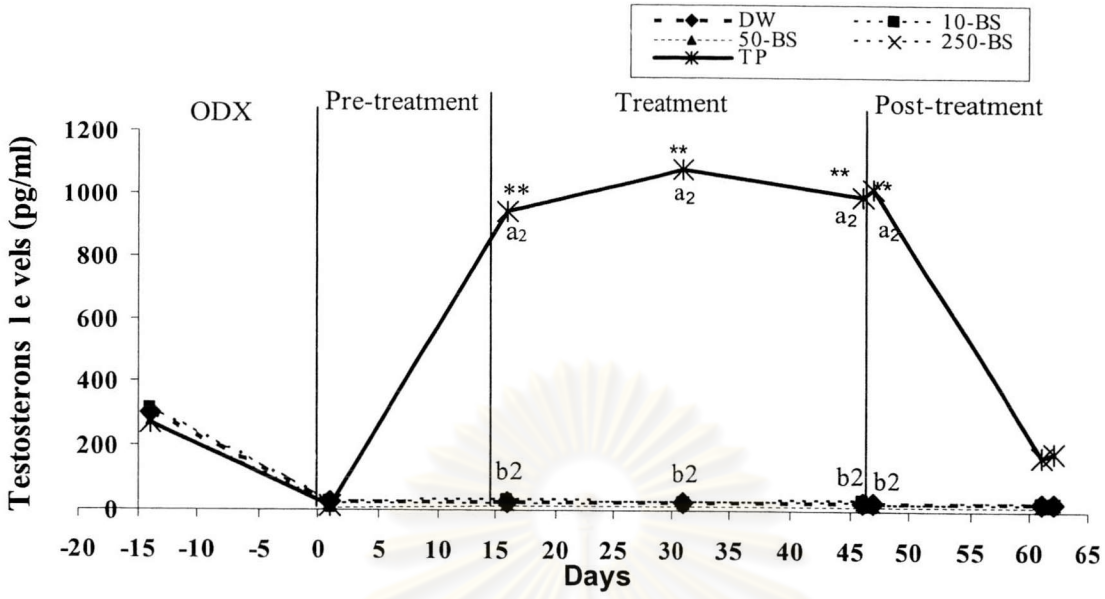


Figure 35. Serum T levels in orchidectomized rats treated with distilled water, *B. superba*, and testosterone propionate.

** = P<0.01 compared to the pre-treatment levels (day 1)

a_2 = P<0.01 compared to DW group

b_2 = P<0.01 compared to TP group

Serum FSH levels

After orchidectomy for 14 days, the serum FSH levels were significantly increased in all 5 groups (5.28 $\pm$0.32ng/ml at day -14 and 26.41$\pm$2.88 ng/ml at day 1, respectively. When compared to the pre-treatment levels (day 1), serum FSH level in DW and BS groups were significantly increased throughout the study period. In TP group, serum FSH levels were decreased between day 31 to day 47 of the study period. The BS groups did not show any significant difference of serum FSH from the DW group in all 3 treatment periods (Figure 36 and Table 12).

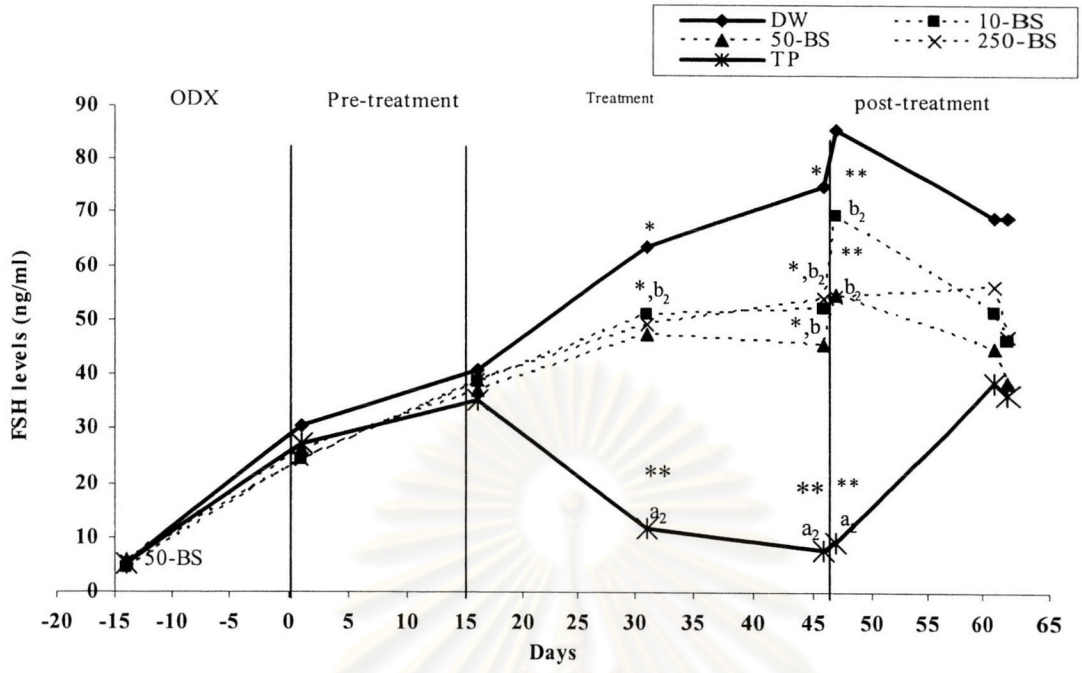


Figure 36. Serum FSH levels in orchidectomized rats treated with distilled water, *B. superba*, and testosterone propionate.

*, **= $P<0.05$ and $P<0.01$ compared to the pre-treatment levels (day 1)

a_2 = $P<0.01$ compared to DW group

b_2 = $P<0.01$ compared to TP group

Serum LH levels

After orchidectomy for 14 days, the serum LH levels were signigicantly increased in all 5 groups. When compared to the pre-treatment levels (day 1), serum LH levels in DW and 10-BS groups were absolutely increased. Serum LH levels at day 16-62 in 50 and 250-BS, except day 46 and 47 in 50-BS, were not significant difference from the pre-treatment levels. Serum LH levels in TP group between day 31-47 were significantly lower than the pre-treatment levels and also highly significantly lower than those of DW and BS groups (Figure 37 and Table 12).

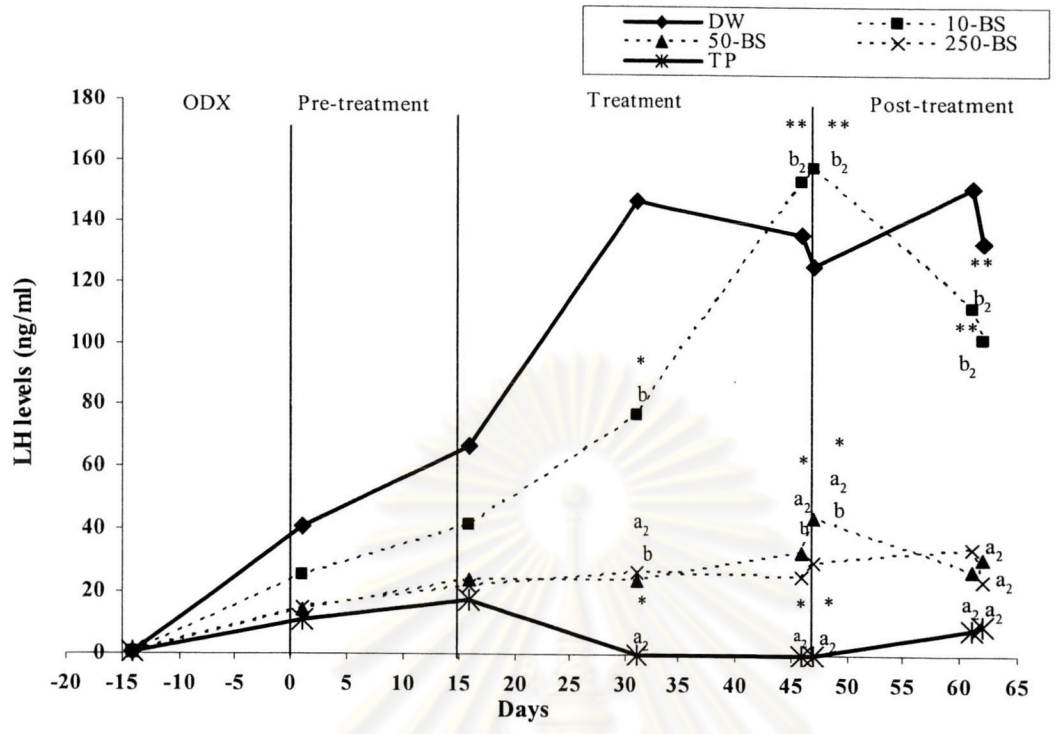


Figure 37. Serum LH levels in orchidectomized rats treated with distilled water, *B. superba*, and testosterone propionate.

*, ** = P<0.05 and P<0.01 compared to the pre-treatment levels (day 1)

a_2 = P<0.01 compared to DW group

b, b_2 = P<0.05 and P<0.01 compared to TP group

The body weights

When compared to the pre-treatment period (day 1), body weights in rat treated with DW, BS and TP were not significantly increased throughout study period.

Also, the body weights in rat treated with BS did not difference from the DW and TP groups (Figure 38).

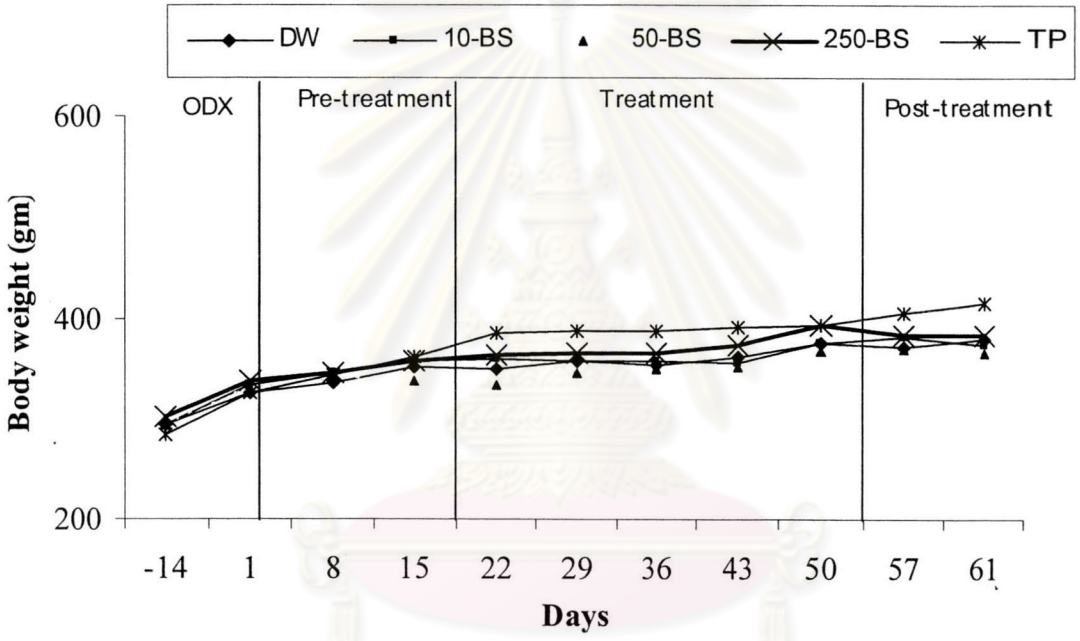


Figure 38. Body weights in orchidectomized rats treated with distilled water, *B. superba*, and testosterone propionate.

The weight and the absolute weight of reproductive organs

Weight and absolute weight of epididymis

When compared the weights of epididymis between the end of treatment and the end of post-treatment periods, no differences were found in all treatment groups, except TP group. The absolute epididymis weights at the end of post-treatment period were higher than at the end of treatment period in DW and all BS groups, and vice versa in TP group.

There were no differences in the weights and the absolute weights of epididymis between DW and BS groups at the end of both periods. However, the weights in TP group were higher than those of BS groups in both periods (Table 13).

	treatment	epididymis weight		absolute epididymis weight (epididymis weight/body weight)	
		end of treatment (gm)	end of post-treatment (gm)	end of treatment ($x10^{-4}$)	end of post-treatment ($x10^{-4}$)
Epididymis	DW	0.121±0.015	0.164±0.016	3.296±0.013	4.082±0.209*
	10-BS	0.191 ± 0.016^{b2}	0.062 ± 0.020^{b2}	2.565 ± 0.108^{b2}	4.201±0.433*
	50-BS	0.127 ± 0.029^{b2}	0.153 ± 0.009^{b2}	2.635 ± 0.110^{b2}	5.400±0.650*
	250-BS	0.103 ± 0.021^{b2}	0.154 ± 0.010^{b2}	2.768 ± 0.560^{b2}	4.088±0.680*
	TP	0.356 ± 0.026^{a2}	$0.282\pm.0.89^{b2,*}$	9.204 ± 0.487^{a2}	6.863±0.508**

Table 13. The weight and the absolute weight of epididymis in orchidectomized rats treated with distilled water, *B. superba* and testosterone propionate.

*, ** = $P<0.05$ and $P<0.01$ compared to the end of treatment period

a_2 = $P<0.01$ compared to DW group

b_2 = $P<0.01$ compared to TP group

Weight and absolute weight of seminal vesicle

When compared the weight and the absolute weight of seminal vesicle between the end of treatment and the end of post-treatment periods, there were no differences in DW and BS groups, but the values at the end of post-treatment period were significantly lower than the end of post-treatment period in TP group.

When compared to the DW group, the weight and the absolute weight of seminal vesicle at the end of both periods were significantly increased in TP group and no differences in all BS groups. The increases of the weight and the absolute weight of seminal vesicle at the end of both periods in TP group were also significantly higher than that of BS groups (Table 14).

	treatment	Seminal vesicle weight		absolute seminal vesicle weight (Seminal vesicle weight/body weight)	
		end of treatment (gm)	end of post-treatment (gm)	end of treatment ($x10^{-4}$)	end of post-treatment ($x10^{-4}$)
Seminal vesicle	DW	0.094±0.010	0.087±0.007	2.631±0.323	2.124±0.145
	10-BS	0.093 ± 0.009^{b2}	0.097 ± 0.007^{b2}	2.633 ± 0.550^{b2}	2.570 ± 0.282^{b2}
	50-BS	0.096 ± 0.004^{b2}	0.085 ± 0.004^{b2}	2.635 ± 0.110^{b2}	2.354 ± 0.172^{b2}
	250-BS	0.102 ± 0.006^{b2}	0.075 ± 0.067^{b2}	2.792 ± 0.173^{b2}	1.996 ± 0.132^{b2}
	TP	3.241 ± 0.155^{a2}	$1.587\pm0.269^{*,a2}$	84.597 ± 5.283^{a2}	$44.388\pm11.354^{**,a2}$

Table 14. The weights and the absolute weights of seminal vesicle in orchidectomized rats treated with distilled water, *B. superba* and testosterone propionate.

*, ** = $P<0.05$ and $P<0.01$compared to the end of treatment levels

a_2 = $P<0.01$ compared to DW group

b_2 = $P<0.01$ compared to TP group

Histological study of reproductive organs

Histology of epididymis

The histology of epididymis in ODX rats treated with DW and BS group showed the absence of stereocilia of ductus tubulus epididymis and the absence of spermatozoa in lumen (Figure 39). Whereas in TP treated group, the tubular epithelium of ductus epididymis is pseudostratified, consisting of tall columnar principal cells with long, stereocilia and small basal cells. There were no difference between the end of treatment group and the end of post-treatment group in DW and BS groups, except the TP group at the end of post-treatment, tubular epithelial all was lower diminished of sterocilia (Figure 40-41).

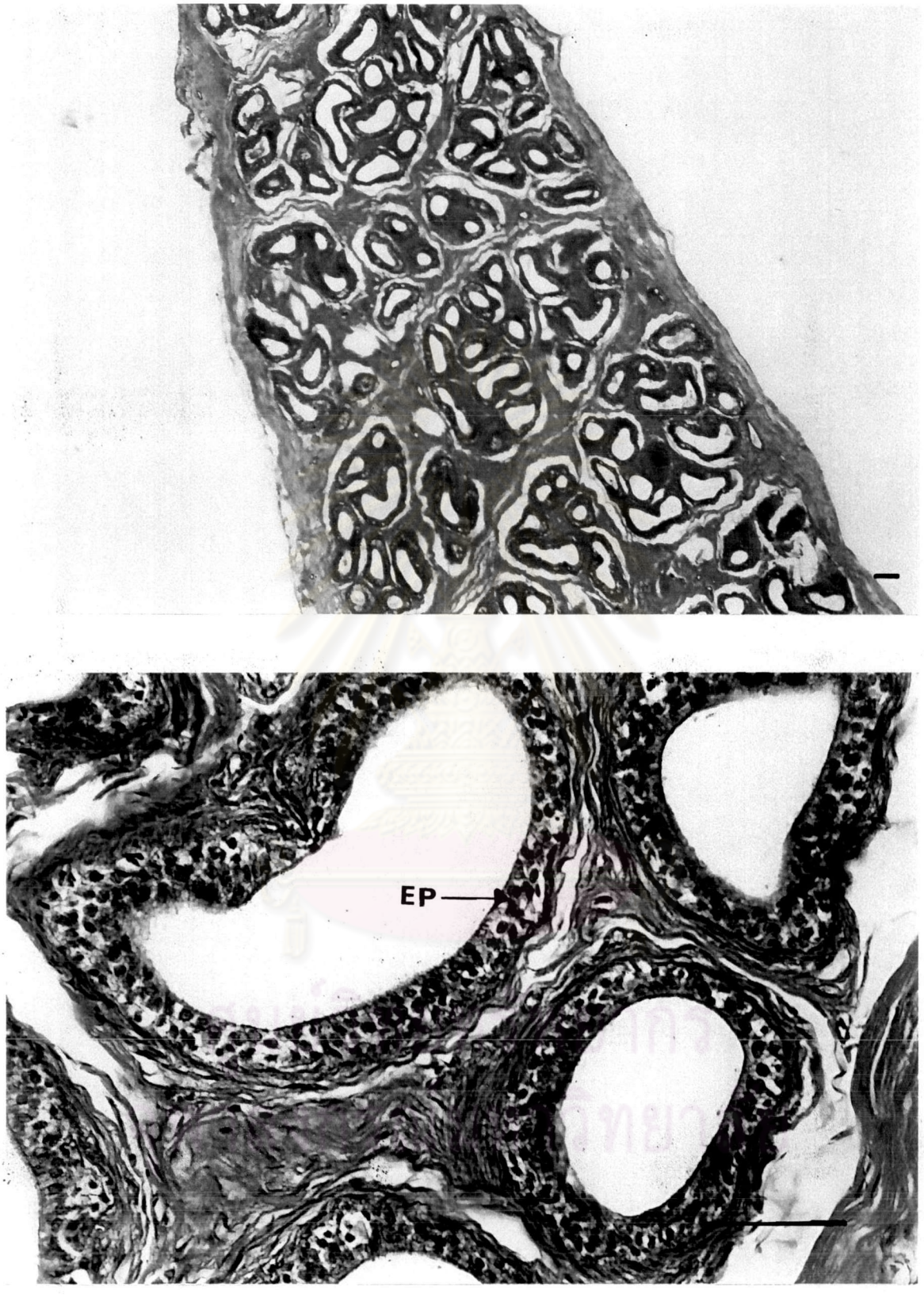


Figure 39. Epididymis morphology in DW group of orchidectomized rats at the end of treatment period. EP=Epithelium. H&E stain. (Scale bars=50 μm)

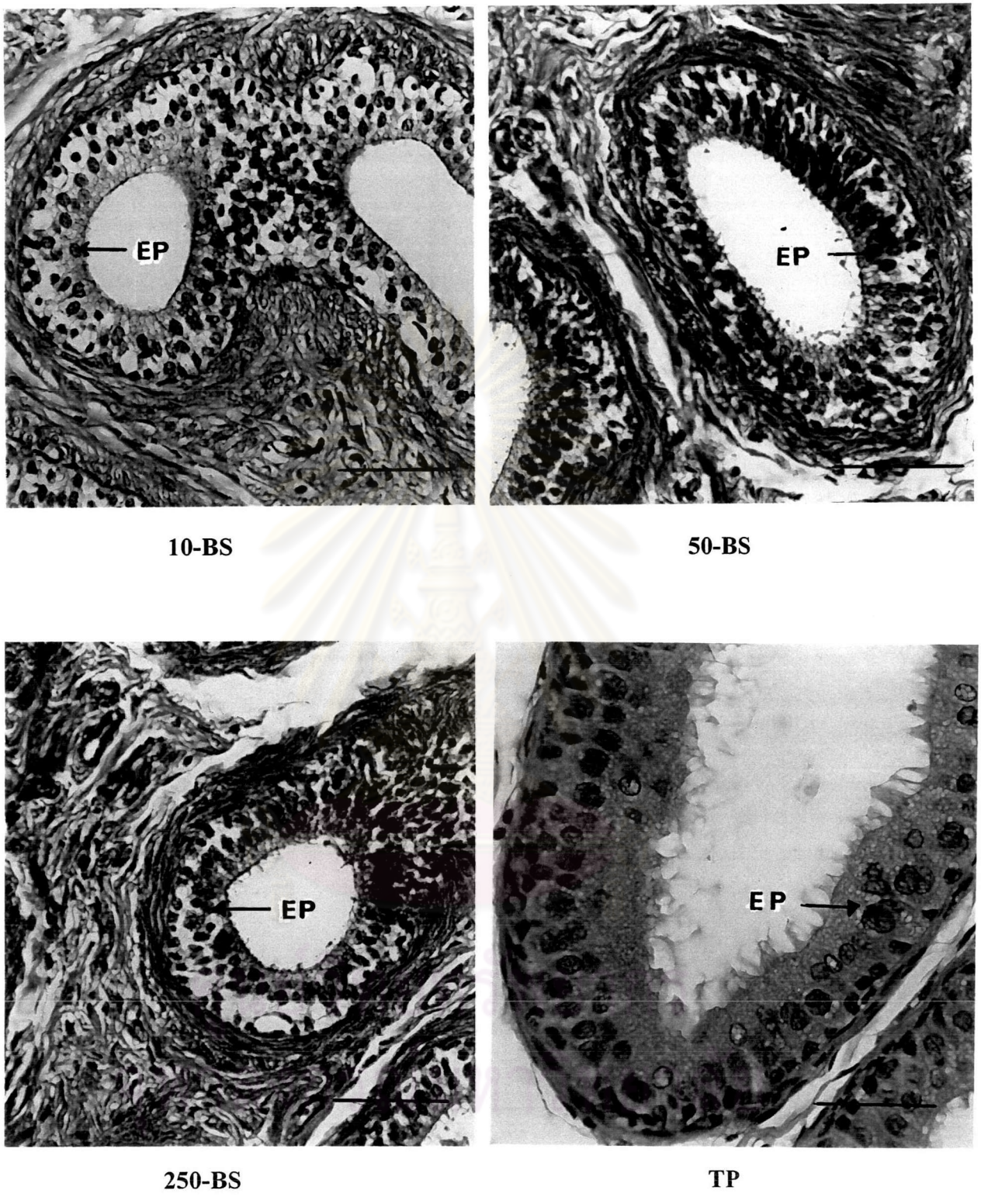


Figure 40. Epididymis morphology in orchidectomized rats treated with 10-BS, 50-BS, 250-BS and TP at the end of treatment period. EP=Epithelium. H&E stain. (Scale bars=50 μm)

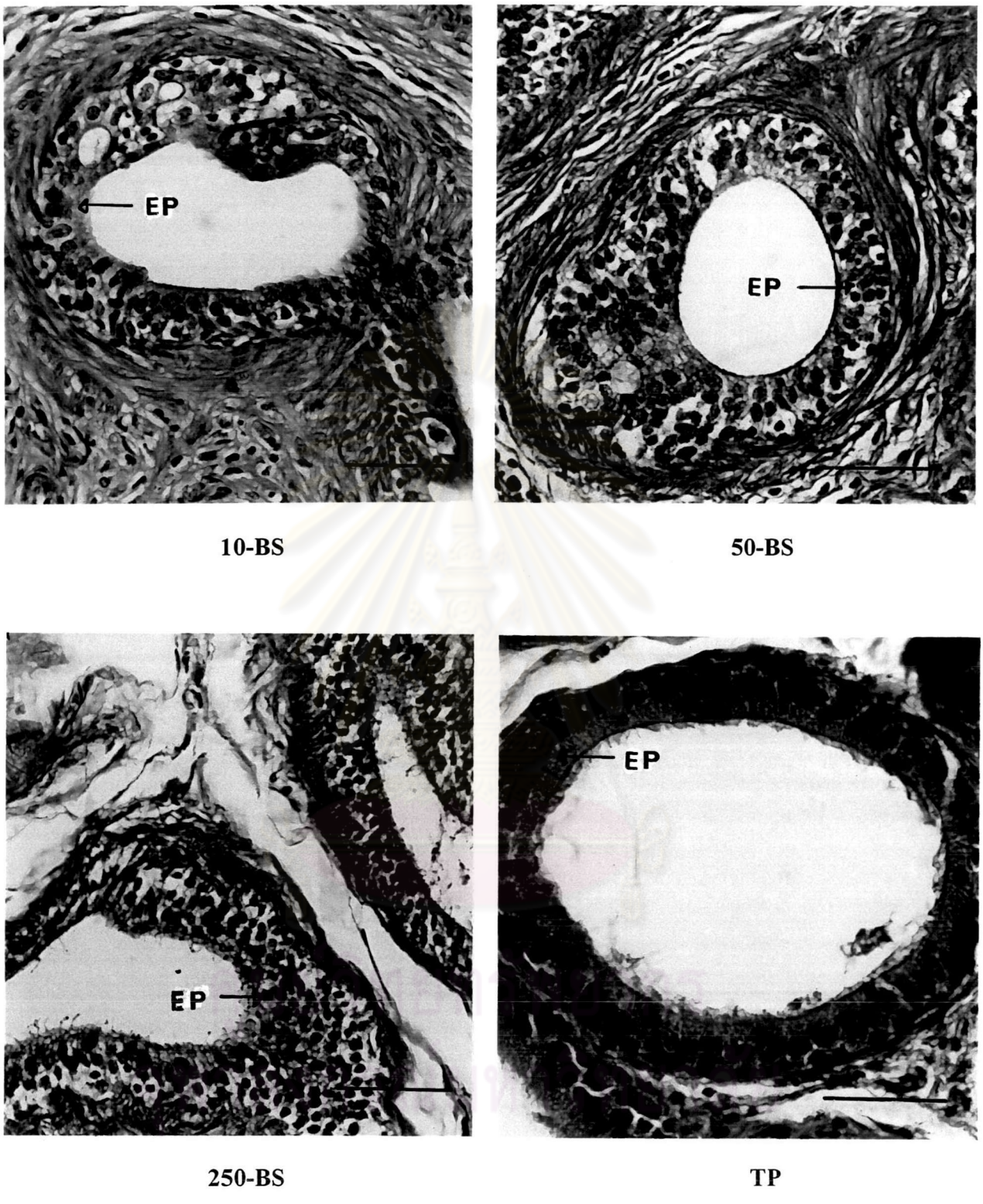


Figure 41. Epididymis morphology in orchidectomized rats treated with 10-BS, 50-BS, 250-BS and TP at the end of post-treatment period. EP=Epithelium. H&E stain. (Scale bars=50 μm)

Histological of seminal vesicle

The histology of seminal vesicle in ODX rats treated with DW and BS groups at the end of treatment period showed the decreasing of epithelial folding pattern. There were absences of seminal vesicle secretion. Whereas, in TP group, there were highly papilla folding pattern of tubular glands. The mucosa composed of numerous primary, secondary and tertiary folding. Furthermore, numerous secretory material were observed in these seminal vesicles. There were no differences between the end of treatment and the end of post-treatment periods in DW and BS groups, but epithelium folding was decreased at the end of post-treatment period in TP group (Figure 42-44).

Figure 42. Seminal vesicle morphology in DW group of orchidectomized rats at the end of treatment period. EX= Extensive folding, EP=Epithelium cell. H&E stain. (Scale bars=50 μm)

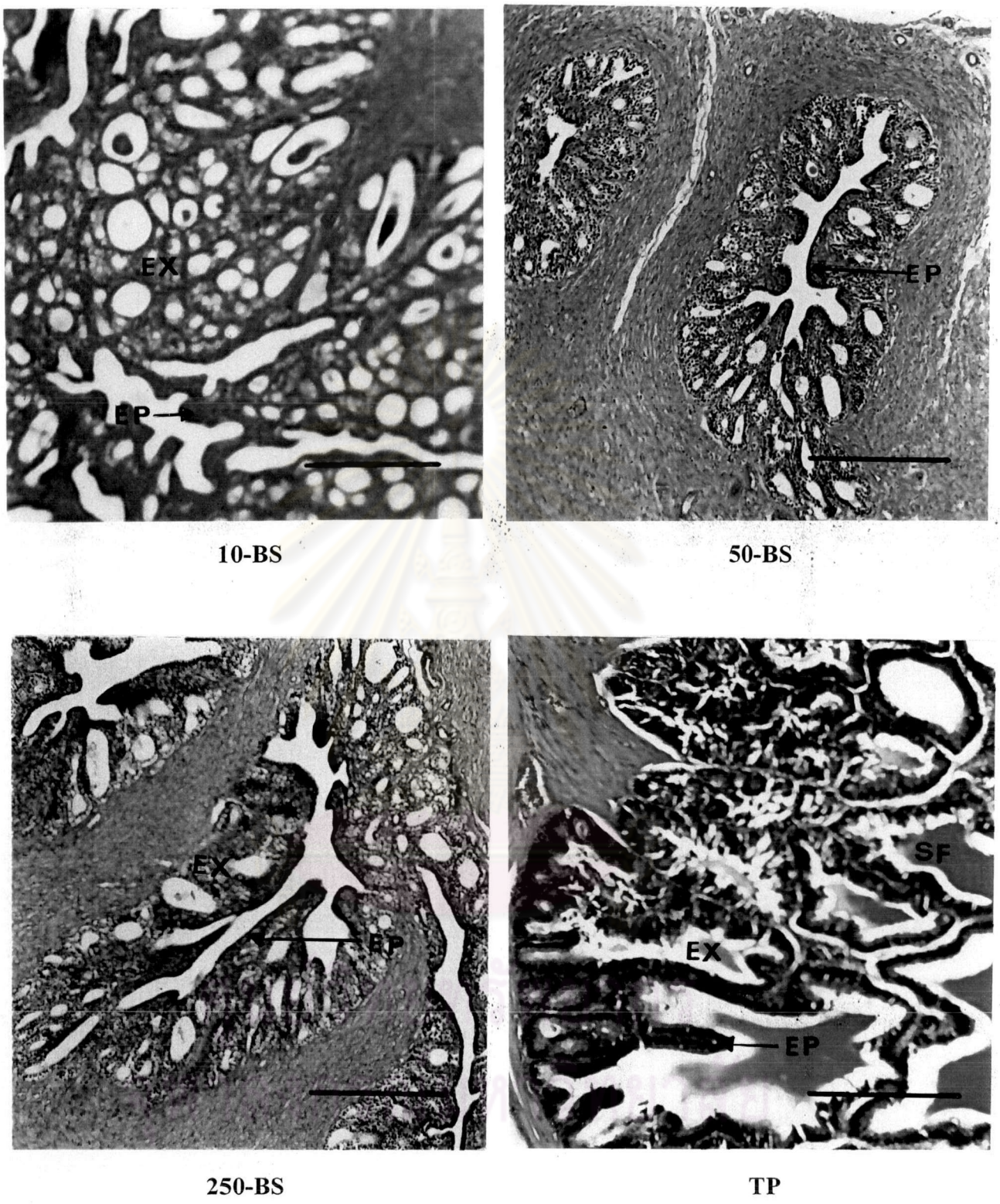


Figure 42. Seminal vesicle morphology in orchidectomized rats treated with 10-BS, 50-BS, 250-BS and TP at the end of treatment period. EX= Extensive folding, EP=Epithelium cell. H&E stain. (Scale bars=50 μm)

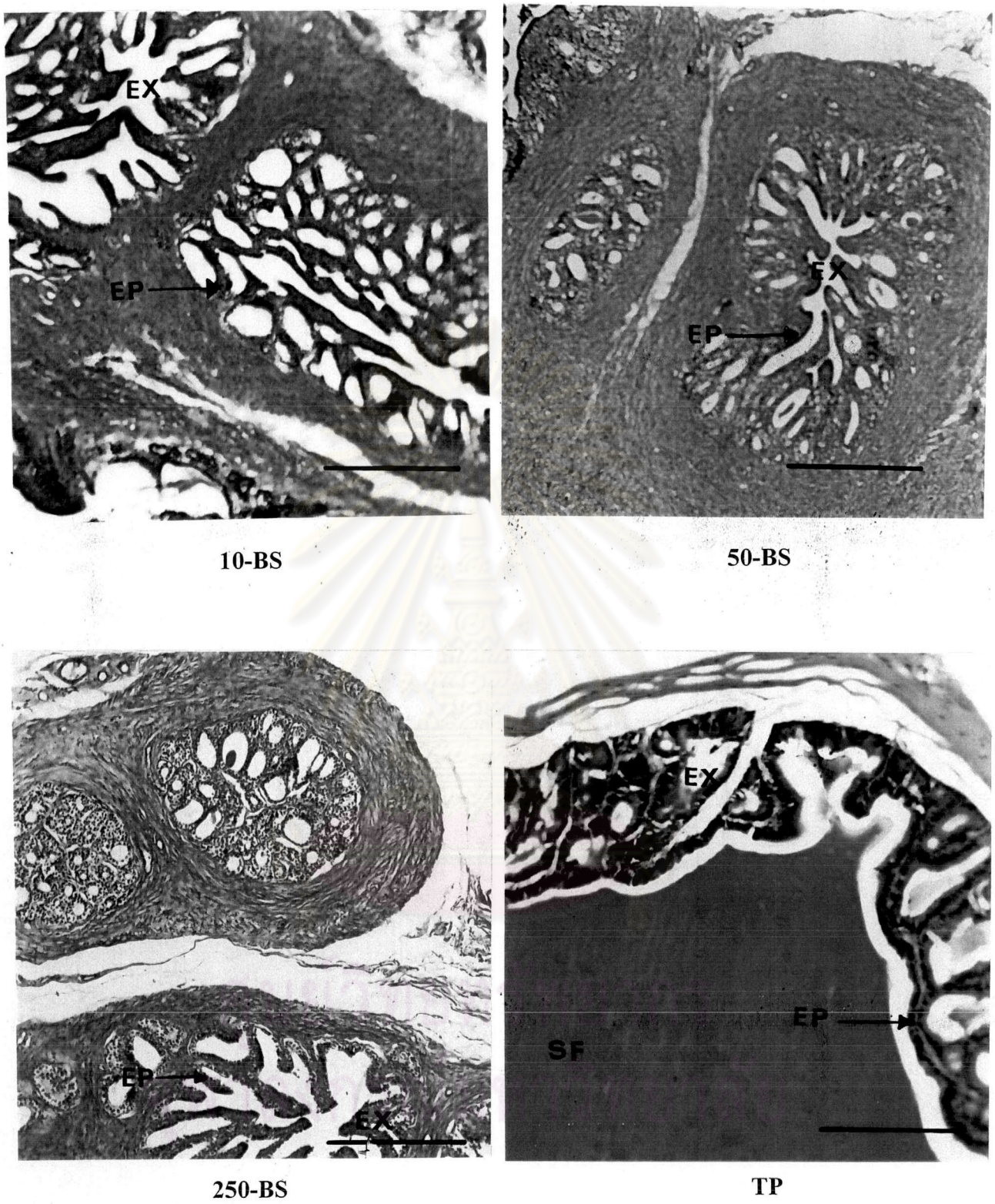


Figure 42. Seminal vesicle morphology in orchidectomized rats treated with 10-BS, 50-BS, 250-BS and TP at the end of post-treatment period. EX= Extensive folding, EP=Epithelium cell. H&E stain. (Scale bars=50 μm)