CHAPTER IV
RESULTS

4.1 Preventive activities of *P. mirifica* and *B. superba* in DMBA-induced rat mammary carcinoma

4.1.1 Growth rate and survival time

The growth rate of rats in the experiment is shown in Figure 4.1 and 4.2. The following data are details of each group.

In *P. mirifica* treated groups, the body weights were increased as found in the control. The initial and final weights are shown in Table 4.1. There were significant difference in body weight of PM-1000 at the 6^{th} -14^{th} and 17^{th}-19^{th} weeks of experiment ($p<0.05$). The others were no significant difference. In BS-1000 group, the growth rate was significant lower than the control at the 6^{th}-8^{th} and 14^{th} week of experiment ($p<0.05$).

In the evaluation for survival, the animals were observed on the first day to the 20^{th} weeks of experiment. The following data are details of each group (Figure 4.3-4.4). No death was found in all rats on the initial week to the 12^{th} weeks of experiment. At the 15^{th} -20^{th} weeks of experiment, the growth of the control was significant declined than PM-100 and PM-1000 group ($p<0.05$). Survival rate of BS-1000 was significant higher than the control at the 16^{th} – 20^{th} weeks of experiment. Others were not observed for difference ($p>0.05$). However, the mean survival times was not found significant different comparing with the control group ($p>0.05$) (Figure 4.5).

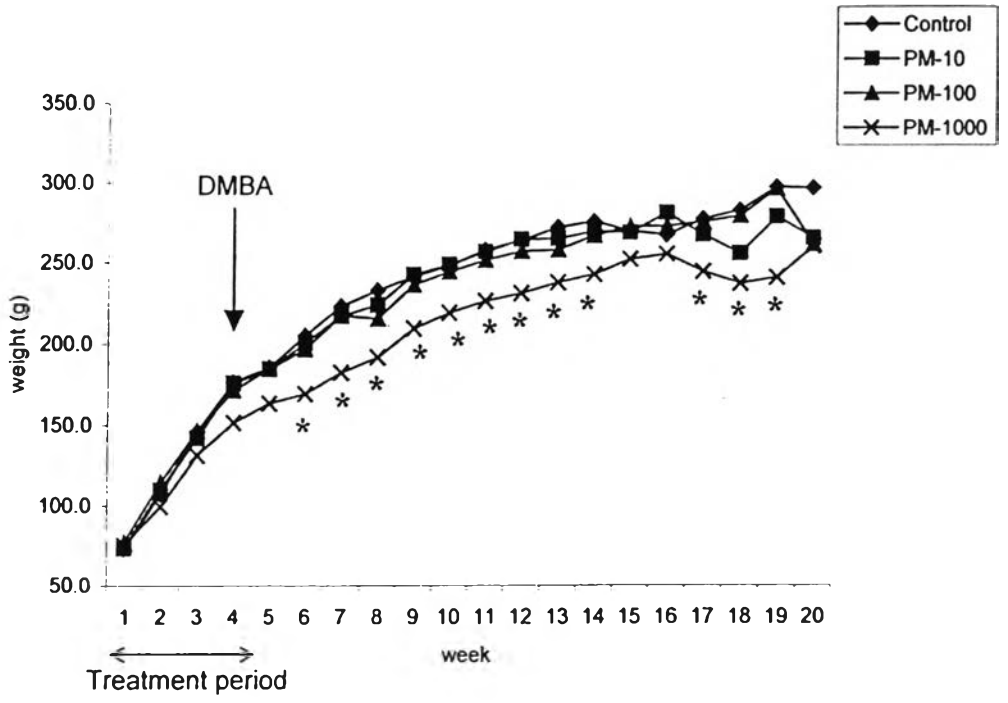


Figure 4.1 Body weight of DMBA-induced mammary tumor rats on preventive study of *P. mirifica*. The asterisk shows significant difference comparing with control at the 0.05 levels.

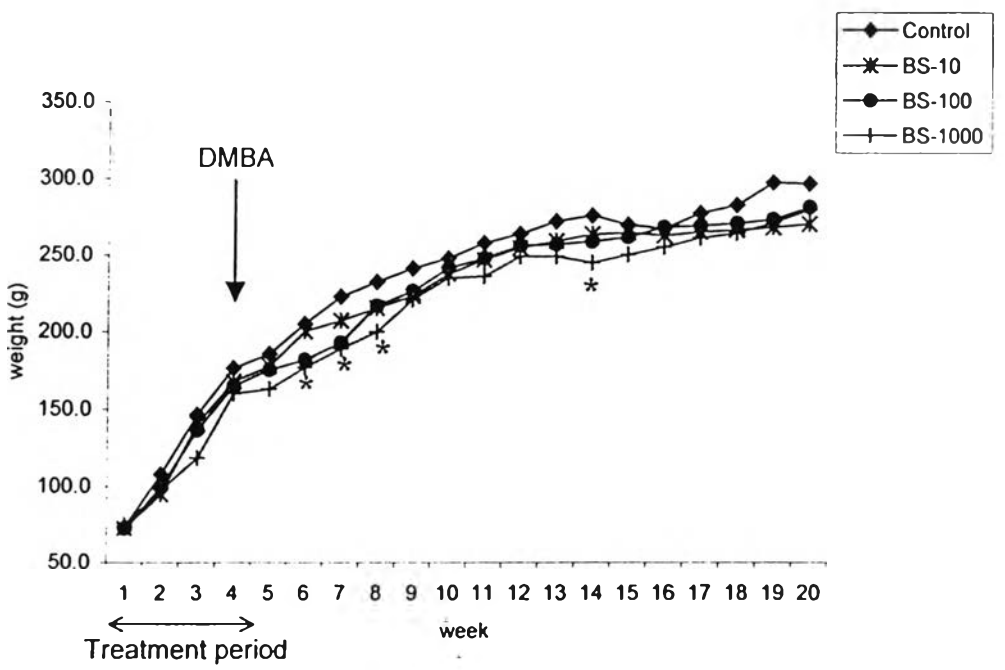


Figure 4.2 Body weight of female DMBA-induced mammary tumor rats on preventive study of *B. superba*. The asterisk shows significant difference comparing with control at the 0.05 levels.

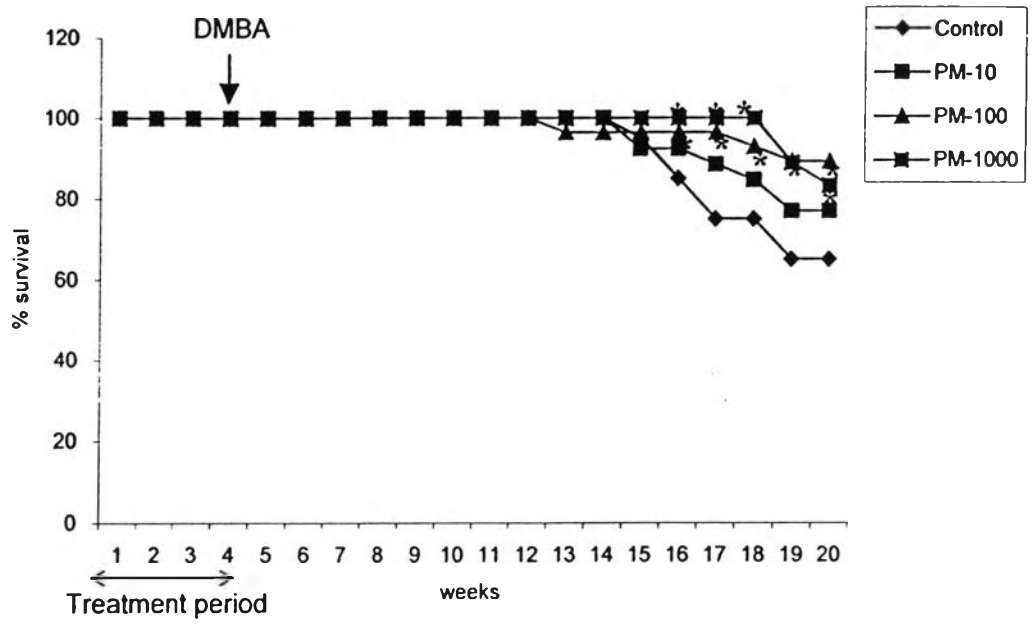


Figure 4.3 Survival rate of DMBA-induced mammary tumor rats on preventive study of *P. mirifica*. The asterisk shows significant difference comparing with control at the 0.05 levels.

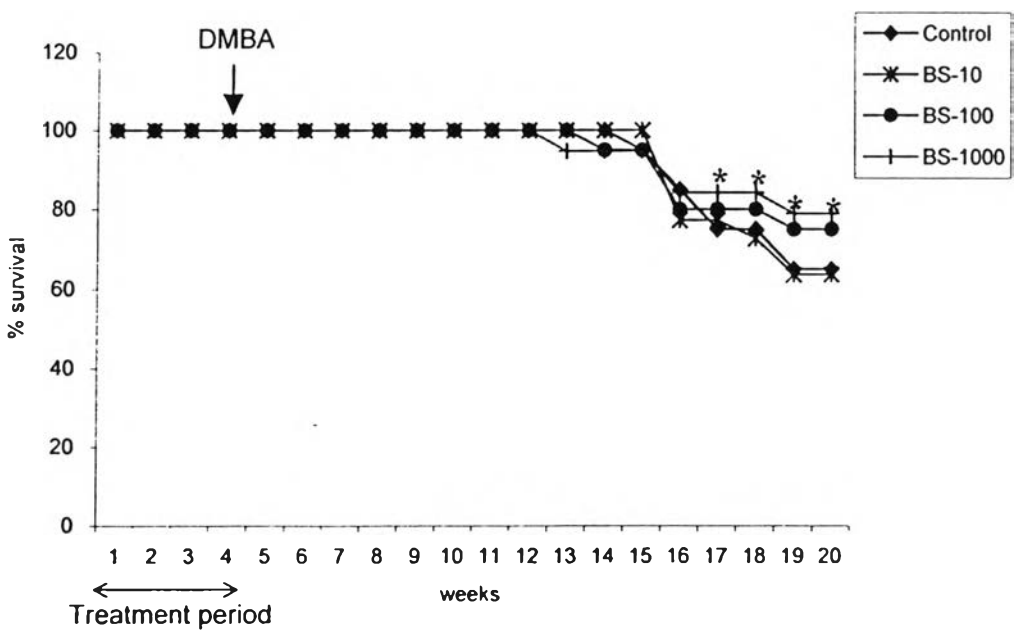


Figure 4.4 Survival rate of DMBA-induced mammary tumor rats on preventive study of *B. superba*. The asterisk shows significant difference comparing with control at the 0.05 levels.

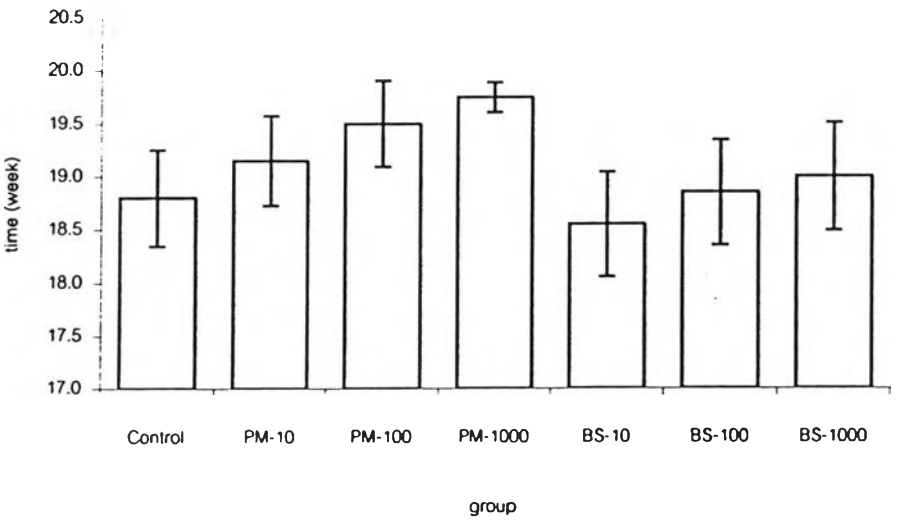


Figure 4.5 Mean survival time of DMBA-induced mammary tumor rats on preventive study of *P. mirifica* and *B. superba*.

4.1.2 Organ

At necropsy day, the rats were weighed and sacrificed. Liver, ovary and uterus weight were recorded (Table 4.1 and Figure 4.5-4.6).

The final body weight of the control was 286.6 $\pm$ 8.6 (Mean $\pm$ S.E.). There was a significant difference in the PM-100 ($p<0.05$) and PM-100 ($p<0.01$) group. For the weight gain comparison, all PM-treated group, BS-10 and BS-1000 were significant lower than the control.

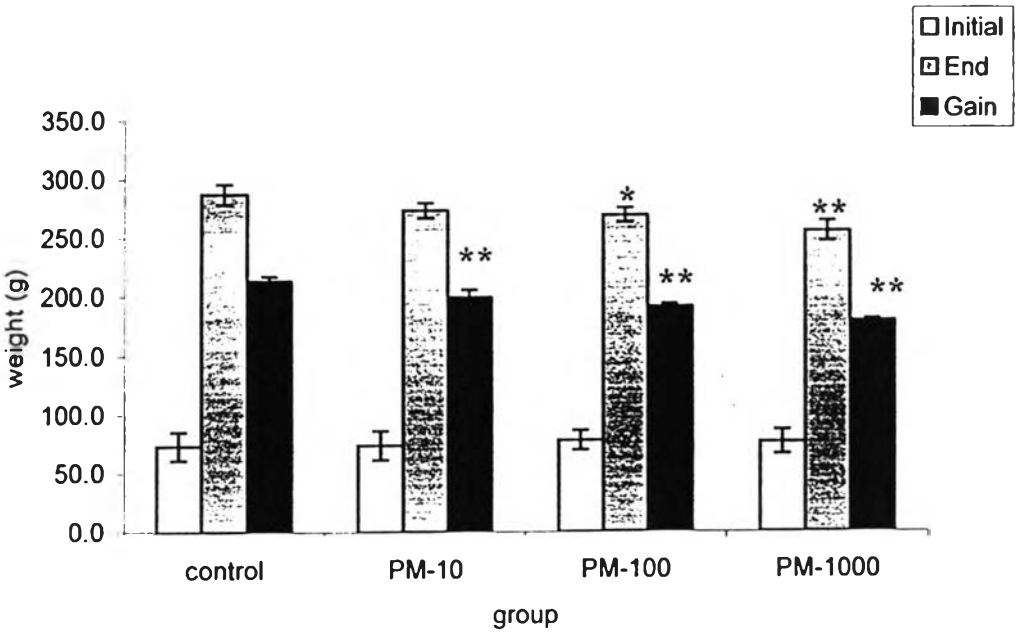


Figure 4.6 Weight gain of DMBA-induced mammary tumor rats on preventive study of *P. mirifica*. An asterisk and double asterisk show significant difference comparing with control at the 0.05 and 0.01 levels, respectively.

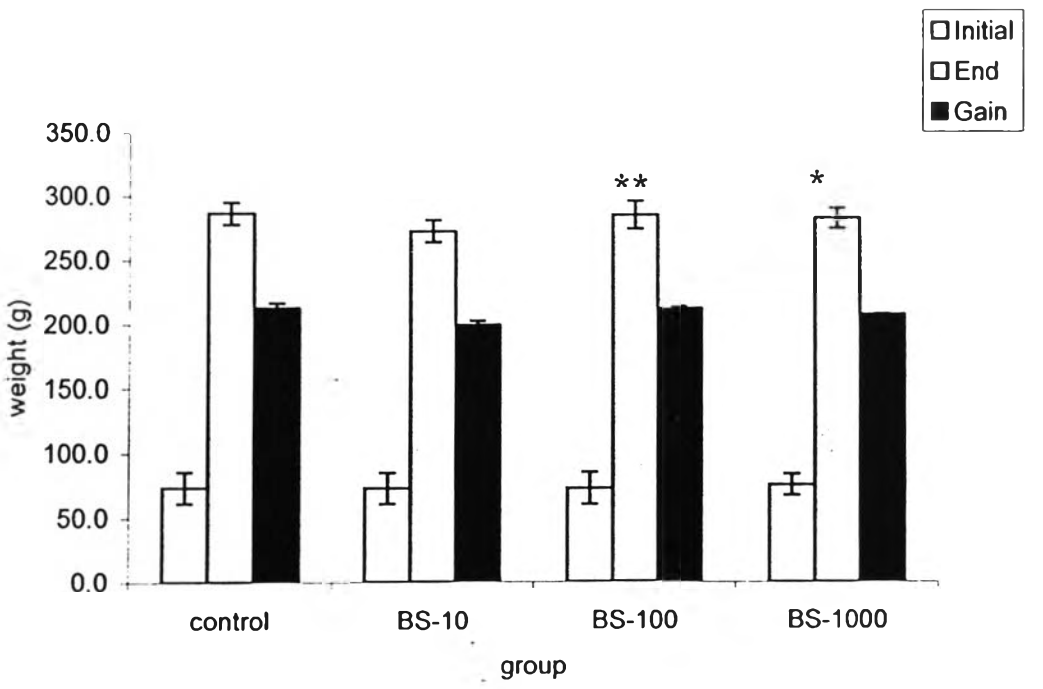


Figure 4.7 Weight gain of DMBA-induced mammary tumor rats on preventive study of *B. superba*. An asterisk and double asterisk show significant difference comparing with control at the 0.05 and 0.01 levels, respectively.

Table 4.1 Final body and organ weight of DMBA-induced mammary tumor rats on preventive study of *P. mirifica* and *B. superba* (Mean ± S.E.).

	Control	PM-10	PM-100	PM-1000	BS-10	BS-100	BS-1000
Body weight (g.)							
Initial	73.5 ± 12.1	73.8 ± 12.4	77.7 ± 8.3	76.0 ± 10.3	72.8 ± 12.0	72.6 ± 12.4	75.3 ± 8.1
Final	286.6 ± 8.6	272.5 ± 6.4	267.9 ± 6.1*	253.2 ± 8.7**	272.3 ± 5.9	284.3 ± 10.8	282.2 ± 7.8
Gain	213.6 ± 3.5	198.7 ± 6.0**	190.2 ± 2.2**	178.2 ± 1.6**	199.5 ± 3.3**	211.7 ± 1.6	206.9 ± 0.3*
Organ weight (g.)							
Liver	9.08±0.32	9.10±0.32	8.58±0.42	8.08±0.49	7.67±0.26**	7.17±0.26**	7.60±0.43**
Relative liver ($x10^{-2}$)	3.19± 0.12	3.33± 0.09	3.18± 0.11	3.19± 0.14	2.82± 0.07**	2.55± 0.11**	2.74± 0.18**
Ovary	0.44 ± 0.16	0.18 ± 0.03**	0.15 ± 0.08**	0.11 ± 0.08**	0.18± 0.01**	0.15± 0.02**	0.15± 0.06**
Relative ovary ($x10^{-3}$)	1.56± 0.57	0.70± 0.15**	0.55± 0.29**	0.44±0.34**	0.67±0.43**	0.55±0.88**	0.55±0.23**
Uterus	0.56±0.05	0.62±0.03	0.59±0.05	0.52±0.03	0.59±0.05	0.53±0.05	0.71±0.07*
Relative uterus ($x10^{-3}$)	1.98 ± 1.18	2.25 ± 0.86	2.22 ± 1.07	2.10 ± 1.40	2.19 ± 0.67	1.87 ± 1.15	2.53 ± 1.79*
Uterus horn length (c.m.)							
Uterus horn1	5.58 ± 0.22	5.54 ± 0.20	5.38 ± 0.09	5.42 ± 0.56	6.13 ± 0.11	6.39 ± 0.39	7.67 ± 0.62**
Uterus horn2	5.37 ± 0.21	5.86 ± 0.23	5.30 ± 0.15	5.98 ± 0.25	5.63 ± 0.60	5.57 ± 0.16	6.03 ± 0.22
Mean uterus horn	5.48 ± 0.25	5.70 ± 0.20	5.34 ± 0.09	5.70 ± 0.33	5.88 ± 0.29	6.36 ± 0.39**	7.63 ± 0.62**

Relative organ = organ/BW, *, ** = the mean difference is significant at the 0.05 and 0.01 compared with control, respectively.

1) Liver

The liver weight of DMBA-induced mammary tumor rats treated with *P. minifica* and *B. superba* were recorded (Table 4.1 and Figure 4.8-4.11). The highest and mean ± S.E. value of the PM-10 was at 9.10 ± 0.32 and the lowest mean ± S.E. was 7.17 ± 0.26 g of BS-100 group. The liver weight of the PM-treated group was found to be no difference compared with the control ($p>0.05$). On the other hand, the BS-treated group was significantly lower than the control ($p<0.01$).

The results of the histological examination of tissues taken from the rats treated with *P. minifica* and *B. superba* at grossly day as shown in Figure 4.10 and 4.11, respectively. In the control group, the histological changes revealed some blood filtration (Figure 4.10A). The moderate fatty degeneration was found in the group of PM-10 (Figure 4.10B). PM-100 (Figure 4.10C) and PM-1000 (Figure 4.10D) found fatty degeneration and dilation which in most of the midzonal area.

In the BS-10 group (Figure 4.11B), the hepatocytes of portal triad were found with fatty degeneration. In the BS-100 and BS-1000 group, the hepatics cells were found hydrographic swelling and mild dilation of sinusoids (Figure 4.11C and D). Furthermore, the blood infiltration was also found in the sinusoids of the BS-100 group (Figure 4.11C).

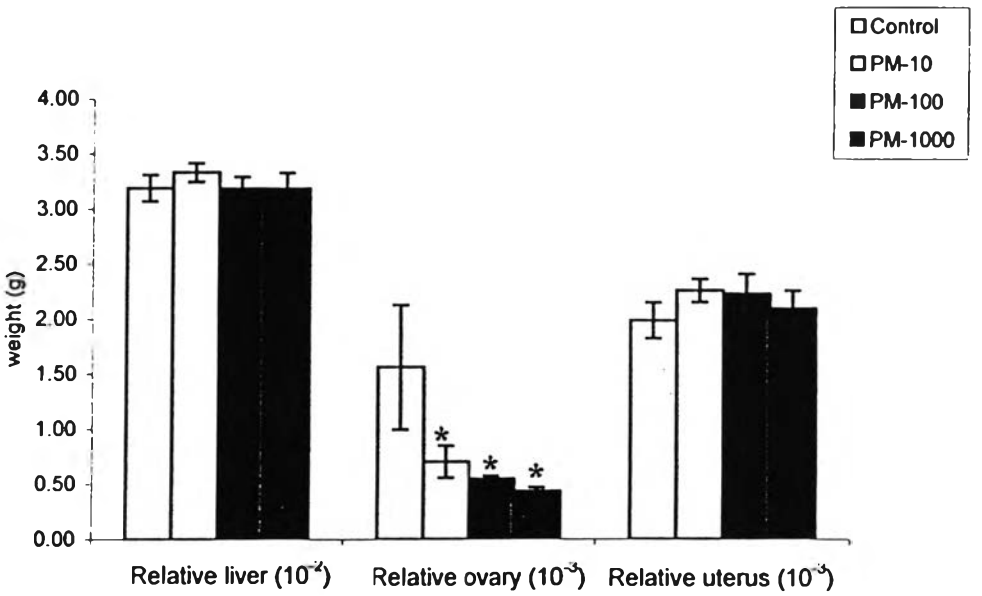


Figure 4.8 Relative liver, ovary and uterus weight of DMBA-induced mammary tumor rats on preventive study of *P. mirifica*. The asterisk shows significant difference comparing with control at the 0.05 levels.

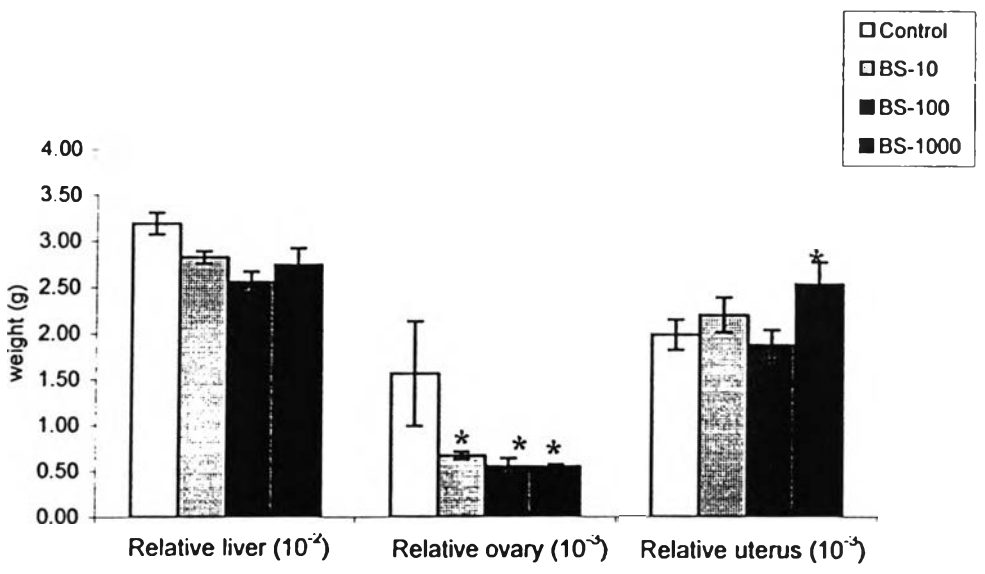


Figure 4.9 Relative liver, ovary and uterus weight of DMBA-induced mammary tumor rats on preventive study of *B. superba*. The asterisk shows significant difference comparing with control at the 0.05 levels.

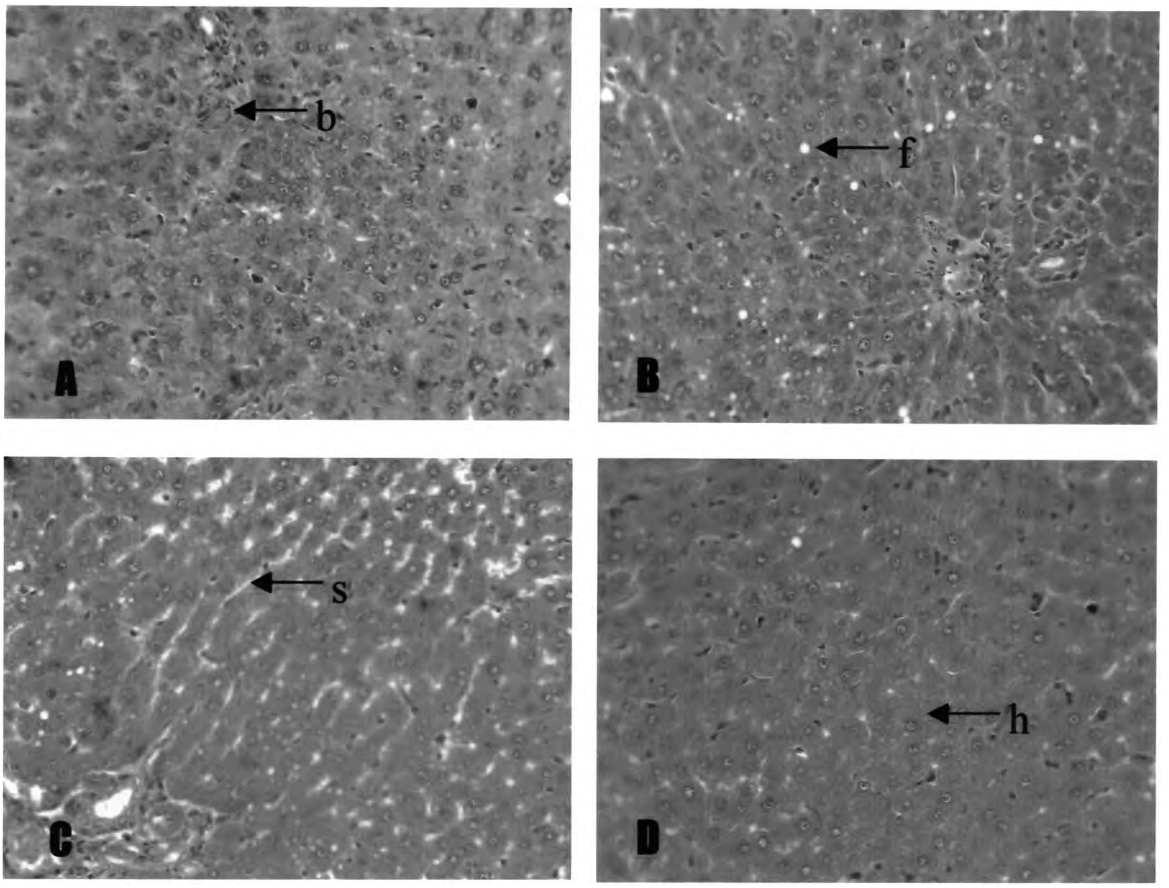


Figure 4.10 Liver tissues of DMBA-induced mammary tumor rats on preventive study of *P. mirifica* (A) control, (B) PM-10, (C) PM-100 and (D) PM-1000. H&E straining for blood filtration (b), fatty degeneration (f), sinusoid dilation (s) and hydrophic swelling (h). Magnitude = x40.

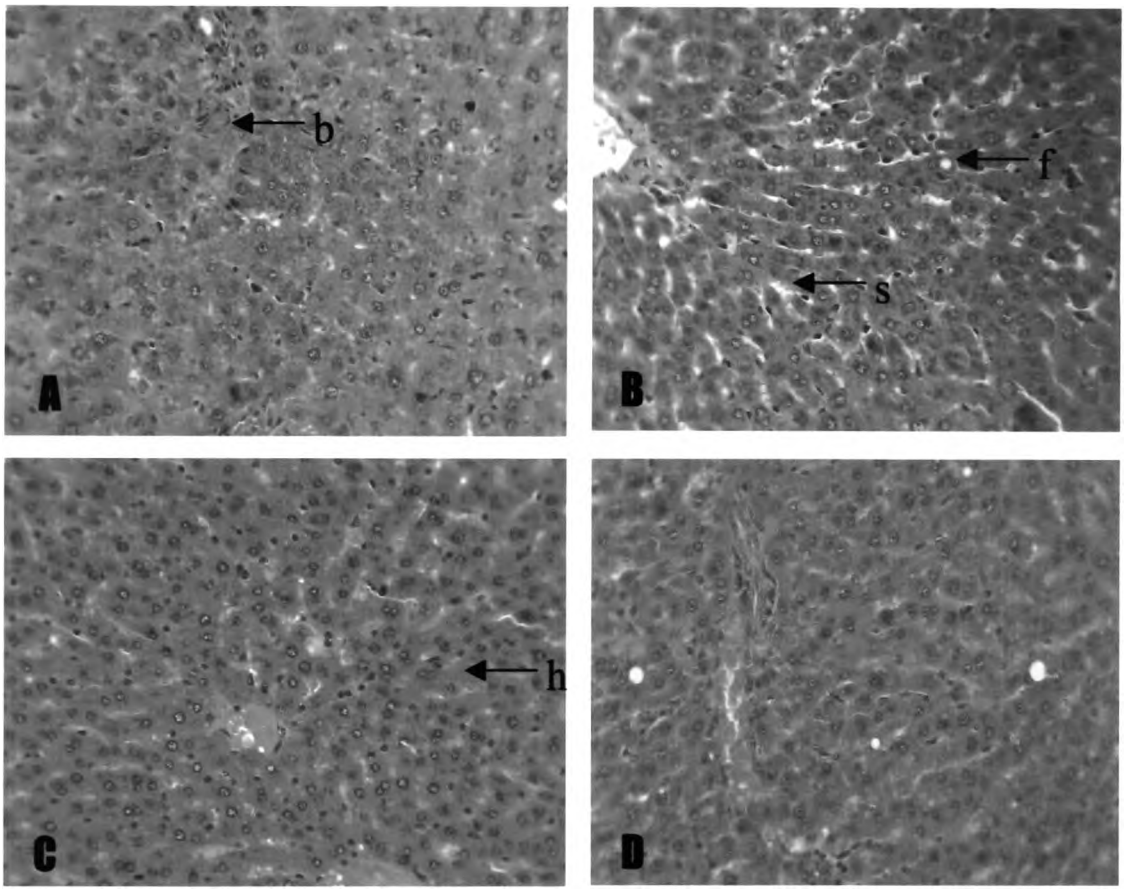


Figure 4.11 Liver tissues of DMBA-induced mammary tumor rats on preventive study of *B. superba*. (A) Control, (B) BS-10, (C) BS-100 and (D) BS-1000. H&E straining for blood filtration (b), fatty degeneration (f), sinusoid dilation (s) and hydrophic swelling (h). Magnitude = x40.

2) Ovary

The weight of the ovary of DMBA-induced mammary tumor in rat treated with *P. mirifica* and *B. superba* were recorded (Table 4.1 and Figure 4.8-4.9 and 4.12-4.13). The highest mean ± S.E. value of ovary weight and relative ovary (ovary weight/BW) was 0.439 ± 0.157 in the control group. Comparison of the ovary and relative ovary weight with control group was found to be significantly difference in all treated group ($p<0.01$).

The histology study of rat ovary in the control, *P. mirifica* and *B. superba* treated group showed the changing of follicles in various phase (Figure 4.12-4.13). In treated groups, Graafian follicle and corpus lutea were clearly found. It was found that were no differences in all group.

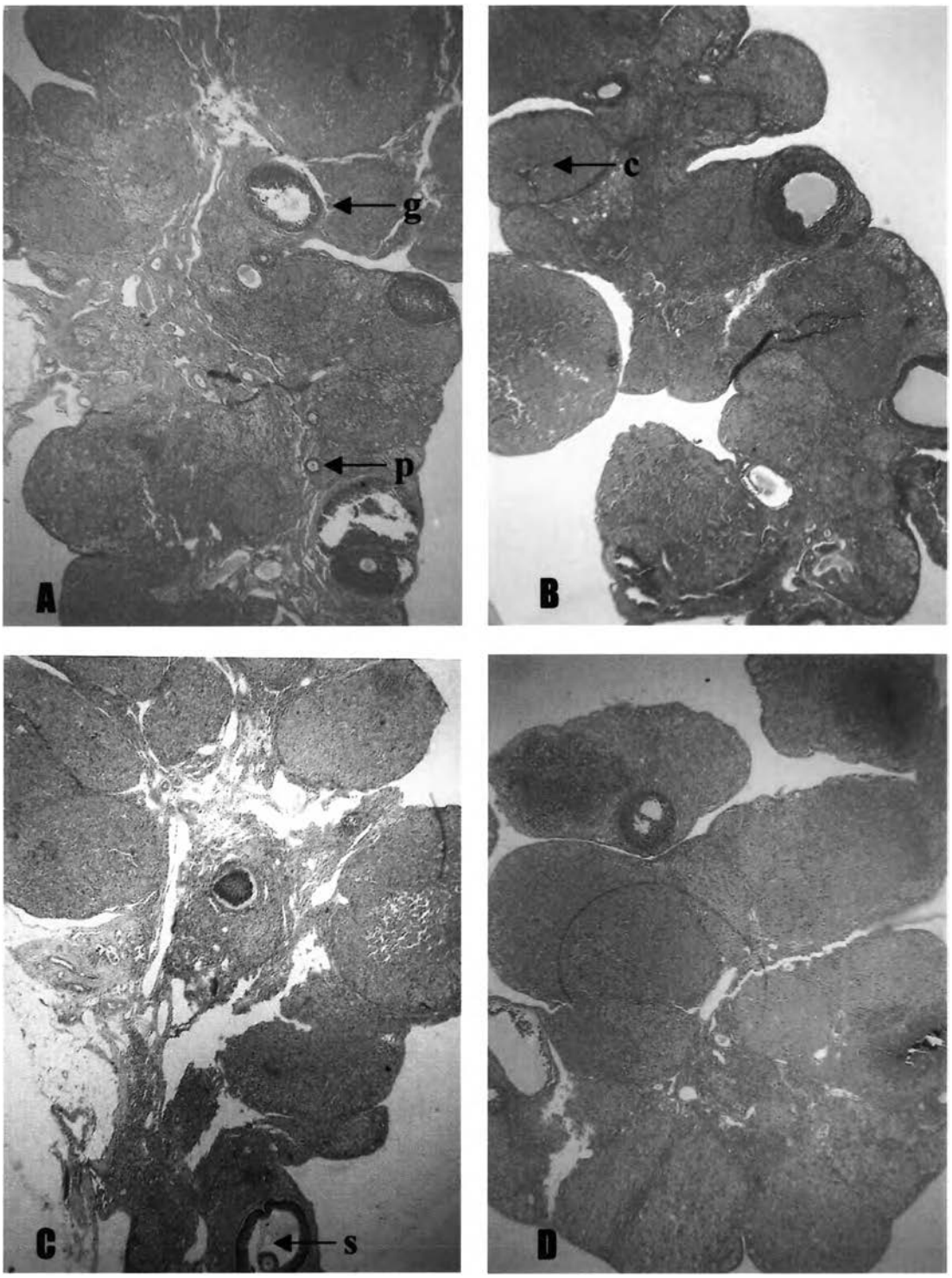


Figure 4.12 Ovary tissues of DMBA-induced mammary tumor rats on preventive study of *P. mirifica*. (A) Control group, (B) PM-10, (C) PM-100 and (C) PM-1000. H&E straining for primary follicle (p), secondary follicle (s), graafian follicle (g) and corpus luteum (c). Magnitude = x10.

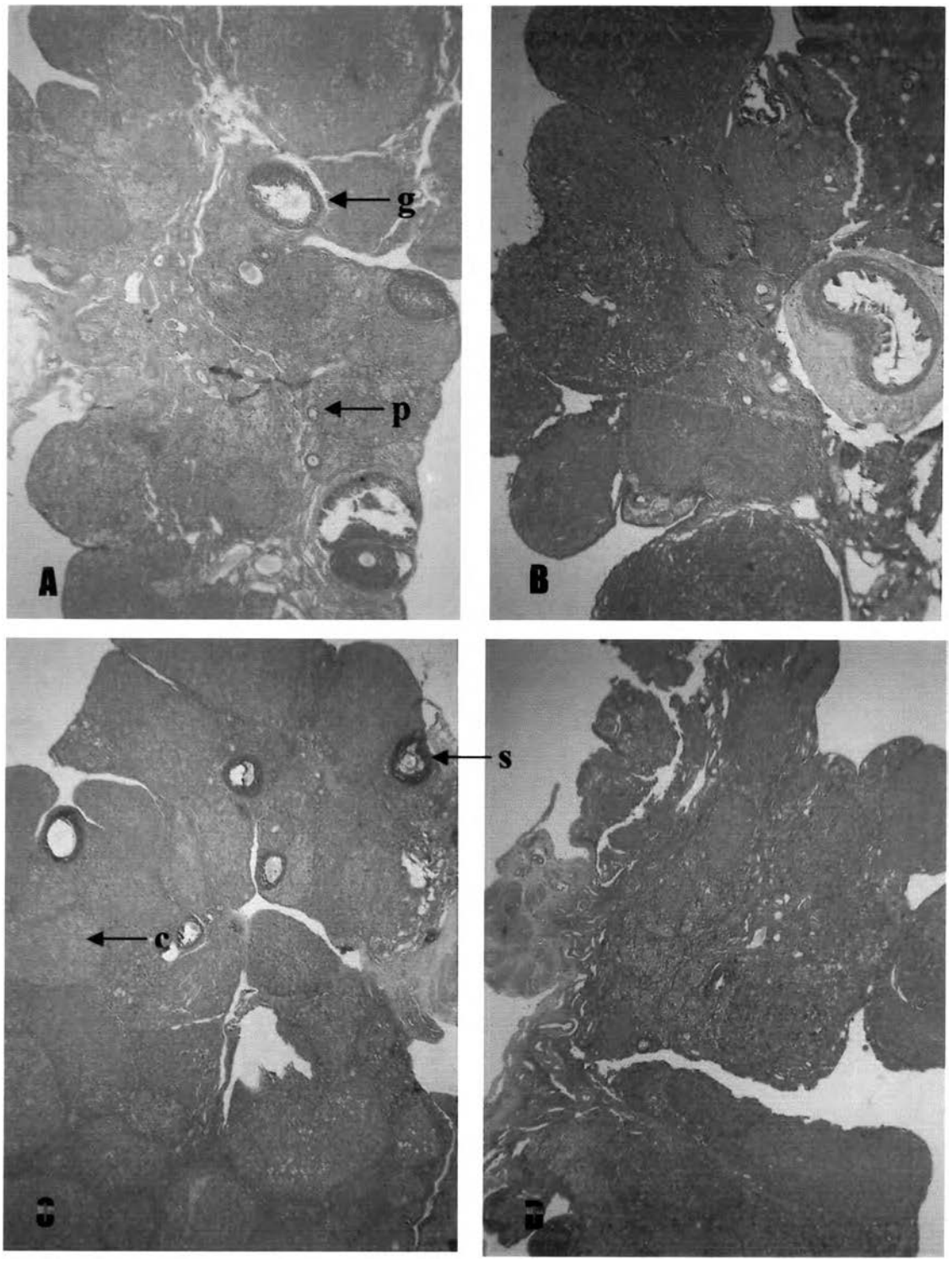


Figure 4.13 Ovary tissues of DMBA-induced mammary tumor rats on preventive study of *B. superba*. (A) Control group, (B) BS-10, (C) BS-100 and (C) BS-1000. H&E straining for primary follicle (p), secondary follicle (s), graafian follicle (g) and corpus luteum (c). Magnitude = x10.

3) Uterus

The weight of uterus and relative uterus of DMBA-induced mammary tumor in rat treated with *P. mirifica* and *B. superba* are recorded (Table 4.1 and Figure 4.8-4.9 and 4.14-4.17). The mean ± S.E. value of the BS-1000 group was significant higher ($p<0.05$) than the control. The length of uterus in BS-100 and BS-1000 group was higher significant than control group at the 0.05 and 0.01 level, respectively.

The histological study of *P. miriifica* treated group was no differences comparing with the control. It is noticed that the mucosa epithelial cell lining and myometrium layer of the BS-1000 group was flatten than control.

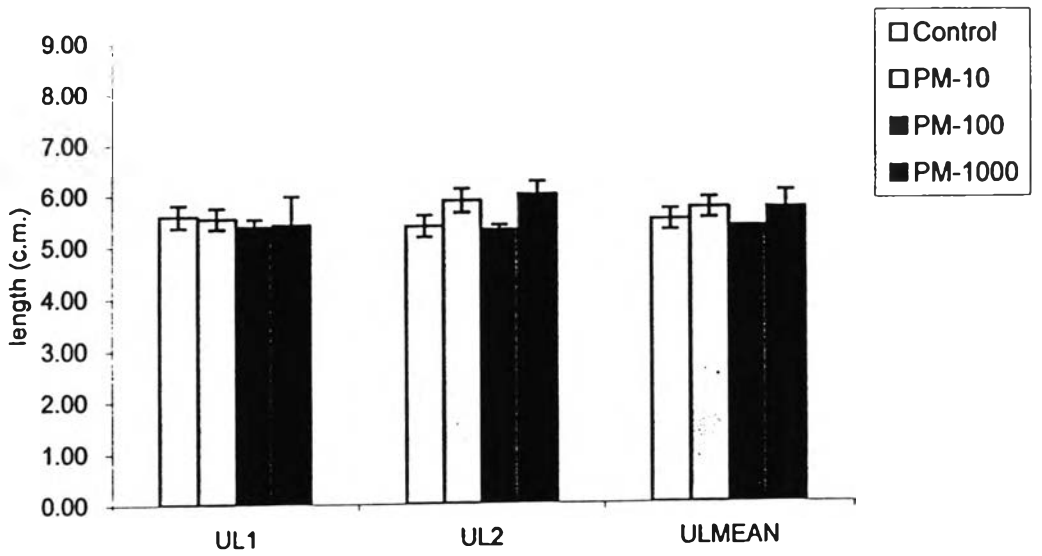


Figure 4.14 Uterus horn length of DMBA-induced mammary tumor rats on preventive study of *P. mirifica* (Mean ± S.E).

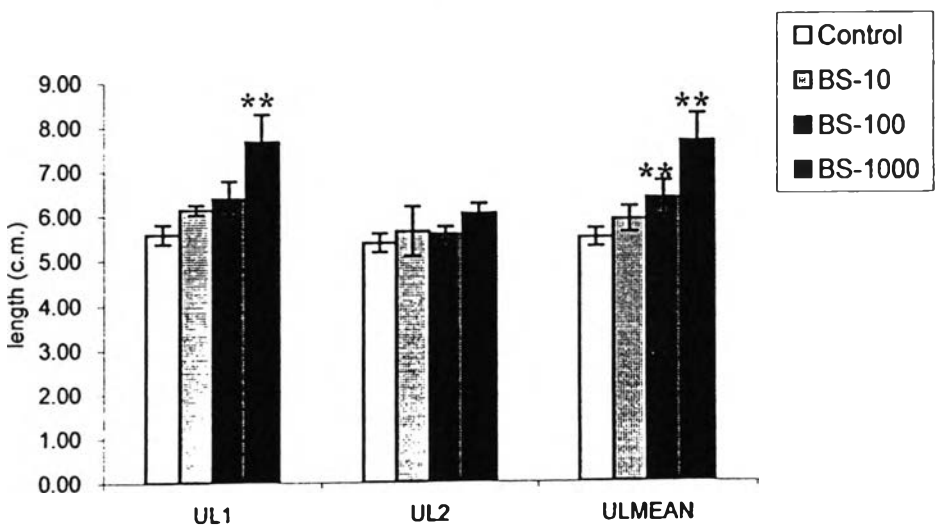


Figure 4.15 Uterus horn length of DMBA-induced mammary tumor rats on preventive study of *B. superba*. Double asterisks show significant difference comparing with control at the 0.01 levels. (Mean ± S.E).

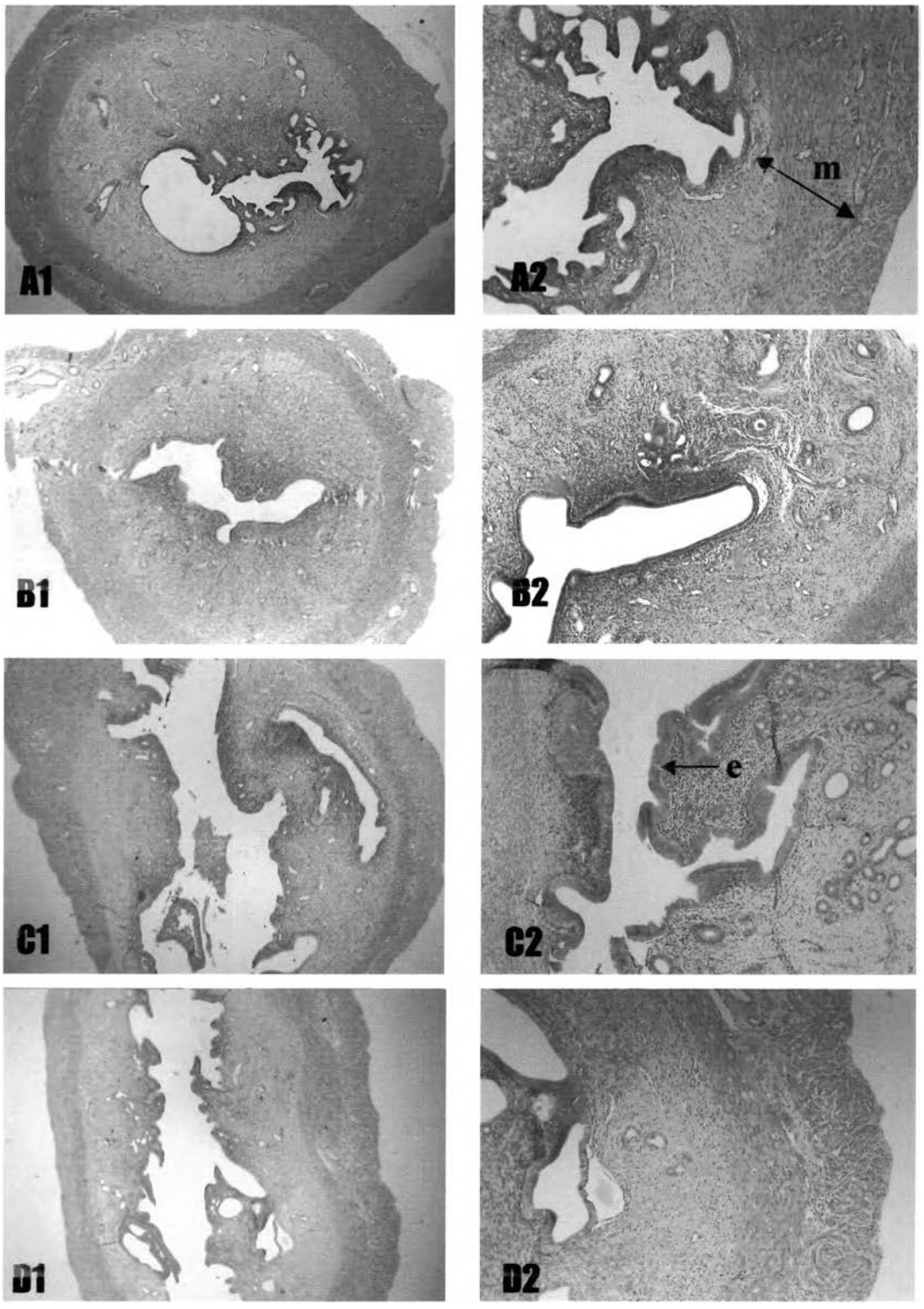


Figure 4.16 Uterus tissues of DMBA-induced mammary tumor rats on preventive study of *P. mirifica*. (A1-2) control group, (B1-2) PM-10, (C1-2) PM-100 and (D1-2) PM-1000. H&E straining for mucosa epithelial (e) and myometrium layer (m). Magnitude of A1, B1, C1, D1 = x10 and A2, B2, C2, 21 = x40.

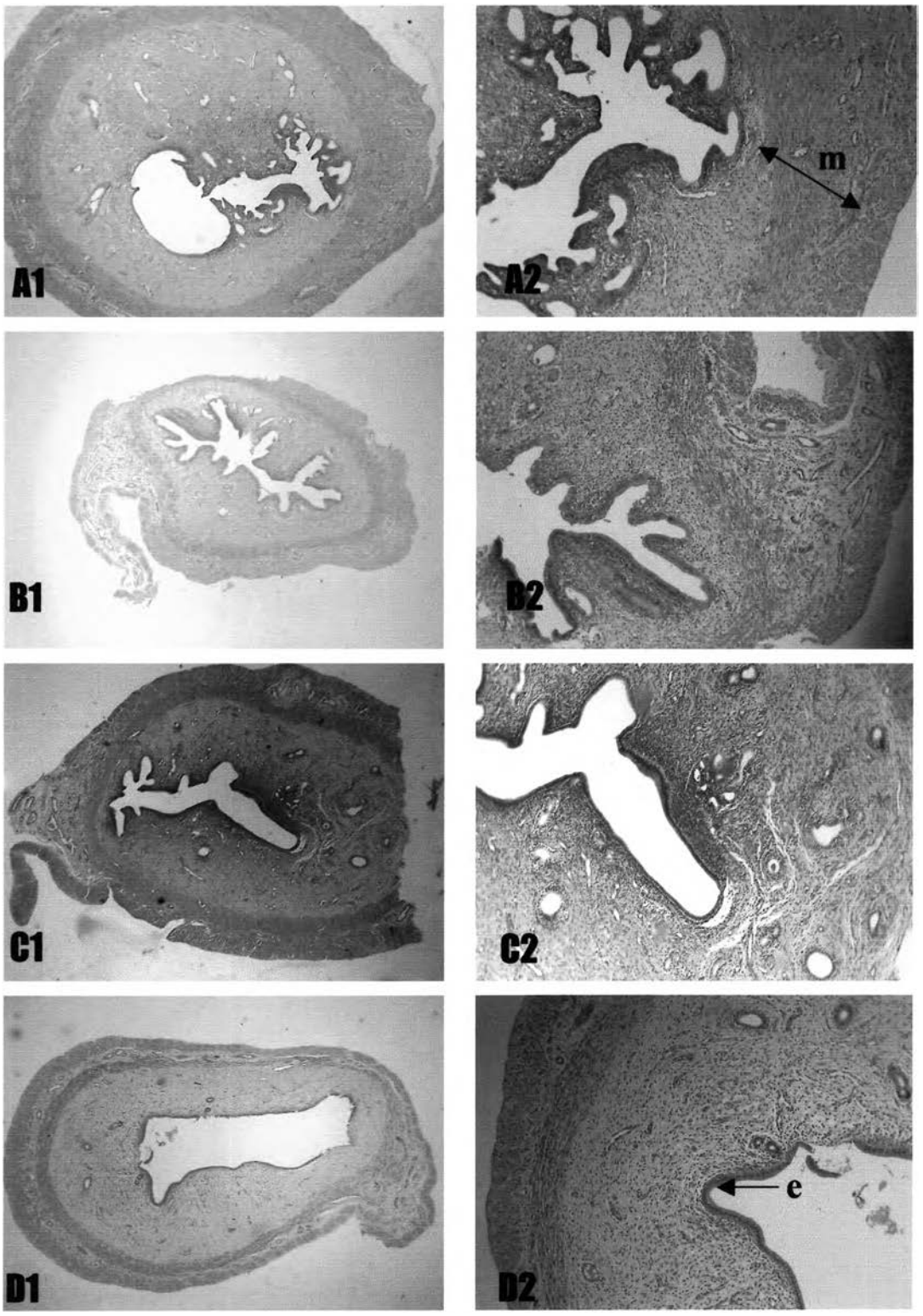


Figure 4.17 Uterus tissues of DMBA-induced mammary tumor rats on preventive study of *B. superba*. (A1-2) control group, (B1-2) BS-10, (C1-2) BS-100 and (D1-2) BS-1000. H&E straining for mucosa epithelial (e) and myometrium layer (m). Magnitude of A1, B1, C1, D1 = x10 and A2, B2, C2, 21 = x40.

4.1.3 Effect of *P. mirifica* and *B. superba* on tumorigenesis

1) Tumor induction and preventive activity evaluation

After treated with *P. mirifica* and *B. superba*, the rats were fed with a single dosage of 80 mg/kg BW of 7,12 DMBA. The rats were palpated for tumor incidence. The first tumor was recorded (Table 4.2 and Figure 4.18-4.19). There was a statistical significant difference between the PM-100, PM-1000, BS-10 and BS-100 compared with the control ($p<0.05$).

The rats were survived and tumor incidences were found between 9^{th} –20^{th} weeks (Table 4.3). The percentage of tumor incidence of the *P. mirifica* treated group was found statistical significant differences in some week. In PM-10, lower tumor incidence in the 9^{th} week of experiment was found. In PM-100, lower tumor incidence in the 9^{th} and 12^{th} week of experiment was found. In PM-1000, lower tumor incidence in the 9^{th}, 12^{th}, 13^{th}, 17^{th} and 20^{th} week of experiment was found. In BS-10 and BS-100, lower of tumor incidence in the 17^{th} and 18^{th} week of experiment were found, respectively. However, there was no significant difference in the BS-1000 group. At the end of experiment, a hundred percentage of tumor incidence in the control and BS-100 treated group was found (Figure 4.20-4.21).

The localization of the first tumor occurrences on DMBA-induced rat mammary tumor was divided similarly with no significant difference ($p>0.5$) (data is not shown).

Table 4.2 The preventive activity of *P. mirifica* and *B. superba* on tumor incidence in DMBA-induced mammary tumor rat.

Group	Time and period of tumor incidence																%Tumor incidence
	Time (week)												Period of incidence (week)				
	9	10	11	12	13	14	15	16	17	18	19	20	Mean	S.E.	Min	Max	
Control	4	0	7	5	1	1	0	0	1	0	0	0	12.95	0.60	9.00	17.00	100
PM-10	0*	2	4	4	0	8*	0	0	0	0	1	0	12.88	0.50	10.00	19.00	95
PM-100	0*	0	3	2	2	6*	0	0	3*	0	2	1	14.43*	0.51	11.00	20.00	96
PM-1000	0*	2	1*	0	0	8*	0	1	4*	1	2	0	15.06*	0.73	10.00	19.00	73*
BS-10	1	4*	1*	4	1	0	0	4*	0	2	3*	0	13.35*	0.70	9.00	19.00	93
BS-100	1	0	5	1	5*	0	3*	3*	3*	0	0	0	12.60	0.61	9.00	18.00	100
BS-1000	4	0	7	5	1	1	0	0	1	0	0	0	13.79*	0.59	9.00	19.00	87

*, ** = The mean difference is significant at the 0.05 and 0.01 levels, respectively.

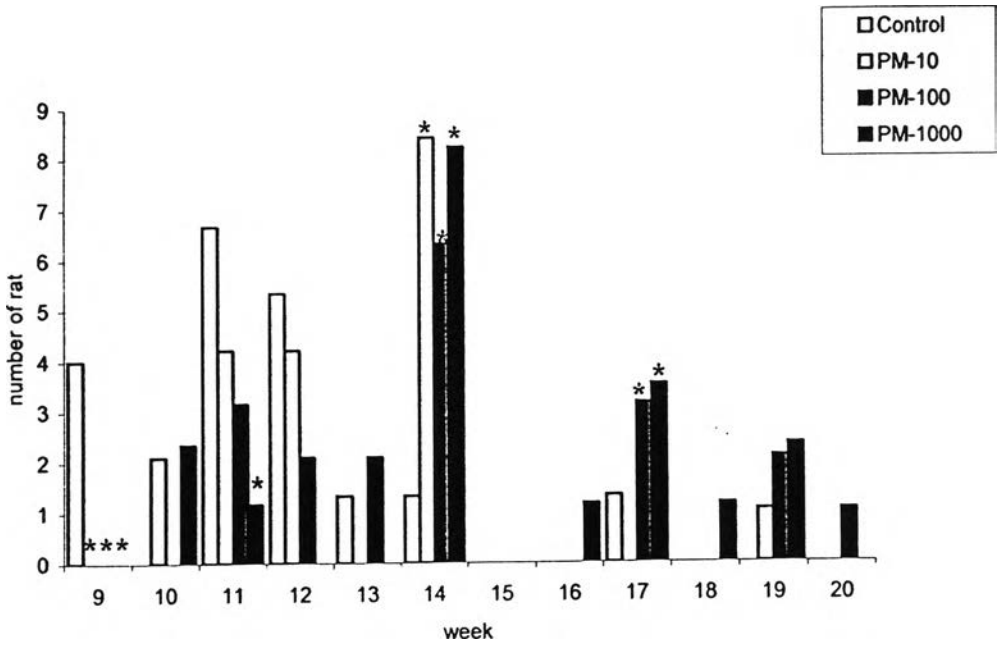


Figure 4.18 Frequencies of rat found with palpable tumor on preventive study of *P. mirifica*. The asterisk shows significant difference compared with control at the 0.05 levels.

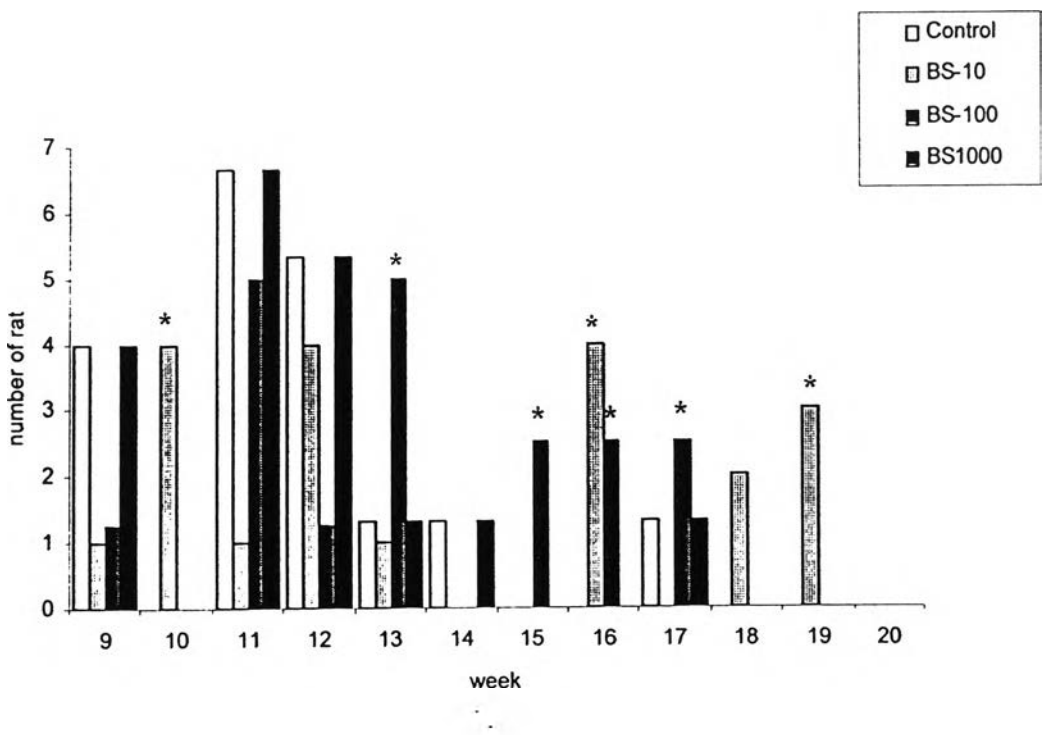


Figure 4.19 Frequencies of rat found with palpable tumor on preventive study of *B. superba*. The asterisk shows significant difference compared with control at the 0.05 levels.

Table 4.3 Accumulative percentage of tumor incidence on preventive study of *P. mirifica* and *B. superba* in DMBA-induced mammary tumor rats.

Week	Control	PM-10	PM-100	PM-1000	BS-10	BS-100	BS-1000
0	0	0	0	0	0	0	0
1	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0
5	0	0	0	0	0	0	0
6	0	0	0	0	0	0	0
7	0	0	0	0	0	0	0
8	0	0	0	0	0	0	0
9	15	0*	0*	0*	5	5	5
10	15	8	0	11	23	15	5
11	40	31	14	17	32	50	26
12	60	50	25*	17**	50	80	32
13	65	50	37	11**	55	85	50
14	70	88	63	50	55	74	50
15	65	88	67	50	55	74	58
16	78	88	67	56	71	81	88
17	94	83	81	67*	71*	75	88
18	94	82	81	72	81	100*	88
19	100	95	92	88	93	100	87
20	100	95	96	73*	93	100	87

*, ** = Statistical significant at $p<0.05$ and 0.01 compared with control, respectively.

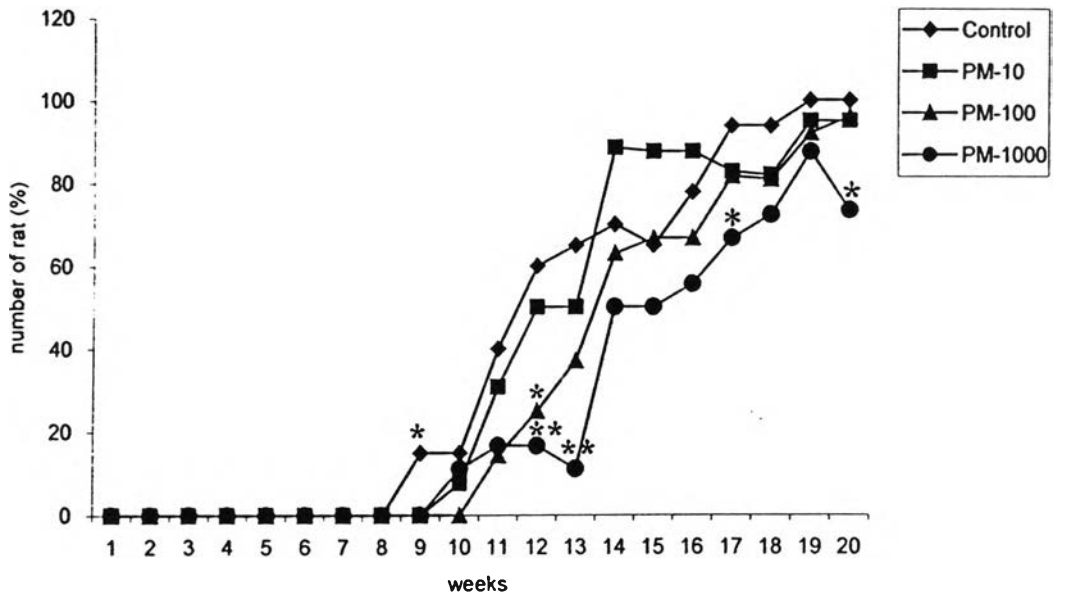


Figure 4.20 Percentages of rat found with palpable tumor on preventive study of *P. mirifica* in DMBA-induced mammary tumor rat. An asterisk and double show significant difference compared with control at the 0.05 and 0.01 levels, respectivly.

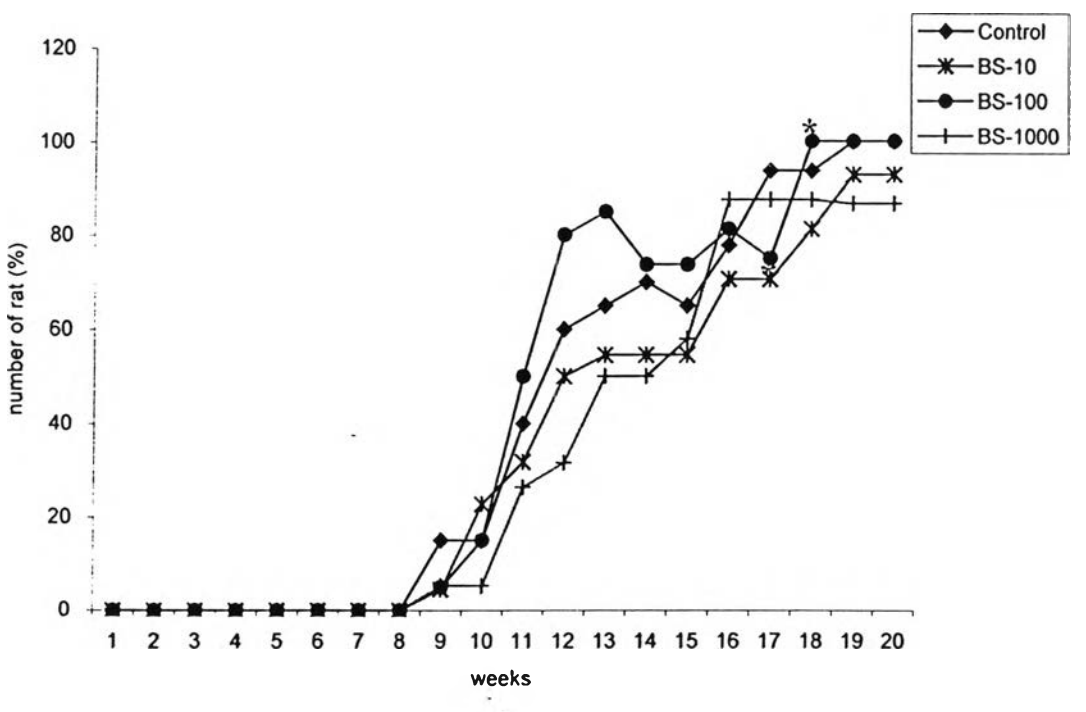


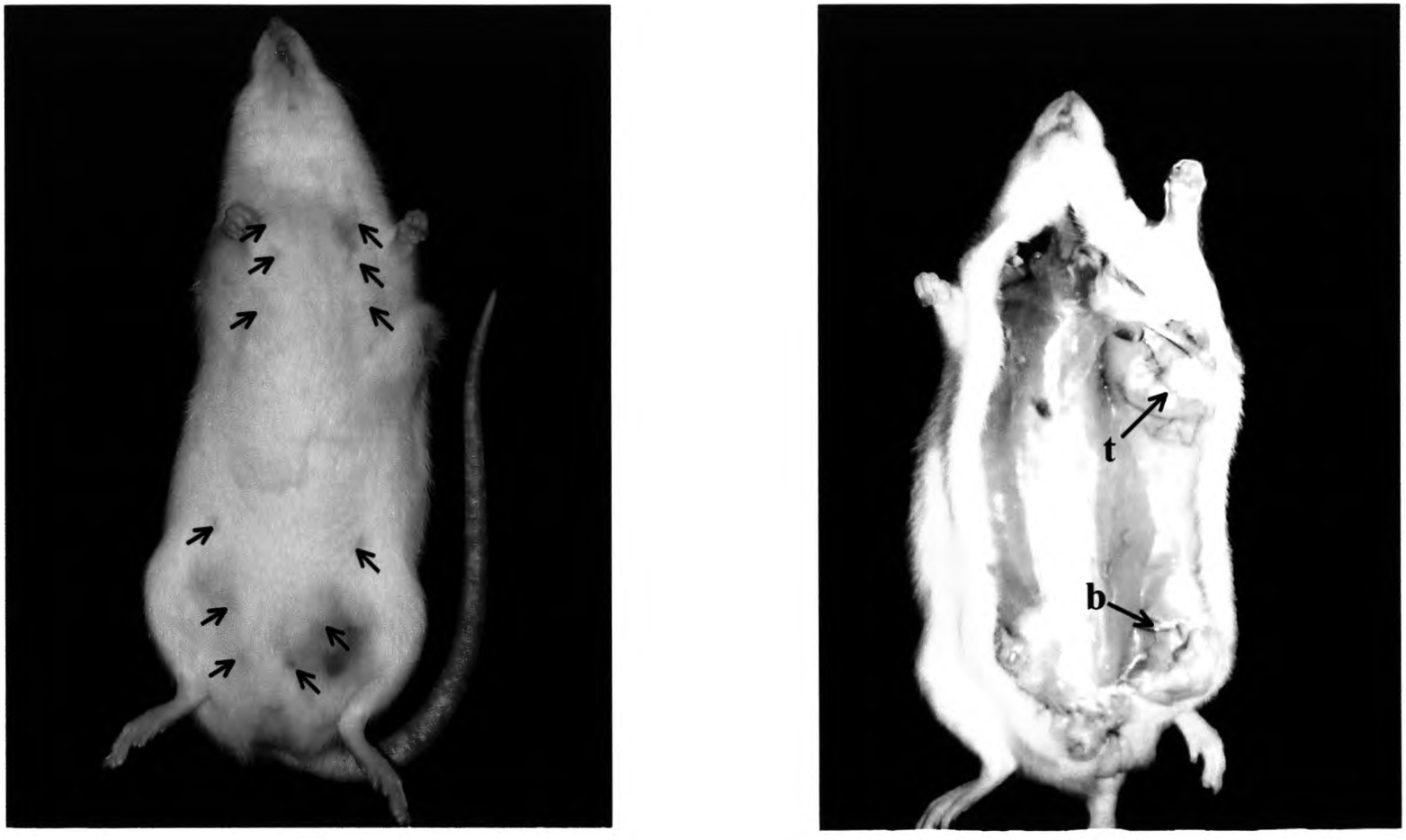
Figure 4.21 Percentage of rat found with palpable tumor on preventive study of *B. superba* in DMBA-induced mammary tumor rat. The asterisk shows significant difference compared with control at the 0.05 levels.

At the grossly day, tumor masses were found distributed in various mammary gland positions which defined into 16 position (Table 4.4 and Figure 4.22-4.24). The pattern of tumor occurrence in both side of the abdomen was recorded (Figure 4.22). The over all tumors at grossly day (n= 20) were recorded (Table 4.44). The tumor distributions were found in all of the 16 positions in the control, PM-10, PM-100 and BS-10 group. In BS-100, BS-1000 and PM-1000 were found in 15, 13 and 13 positions, respectively. The statistical significant difference ($p<0.05$) was shown (Table 4.4) Comparison of the right and left side of the abdomen in each treatment, significant differences in all PM-treated group and BS-1000 group ($p<0.05$) were found (Figure 4.25-4.26).

Table 4.4 Mammary tumor localization on preventive study of *P. mirifcia* and *B. superba* in DMBA-induced rat

No.	Location	Control	PM-10	PM-100	PM-1000	BS-10	BS-100	BS-1000
1	VR0	8	10	3*	3*	8	5	5
2	VR1	8	6	6	5	7	6	9
3	VR2	13	5*	2*	6*	8	13	4
4	VR3	6	5	2*	1*	3*	6	3*
5	VR4	1	5*	3	6*	8*	2	0
6	VR5	5	5	5	0*	3	4	1*
7	VR6	5	1	3	0*	2	1*	4
Total		45	36	24	21	39	36	24
10	VL0	9	7	6	2*	8	6	4
11	VL1	6	6	6	5	6	6	4
12	VL2	5	6	10*	3*	8*	8*	6
13	VL3	5	4	5	4	4	5	6
14	VL4	3	3	3	3	3	2	0
15	VL5	5	2*	4	2*	1*	1*	1*
16	VL6	3	5	2	0*	2	0*	0*
Total		35	33	34	19	32	28	21

* = Statistical significant at $p<0.05$ compared with control.



(A) (B)

Figure 4.22 Tumor appearance on DMBA-induced mammary tumor in rat (A) surface (B) gross. Arrows show mammary nipple of rat, mammary tumor (t) and blood vessel (b).

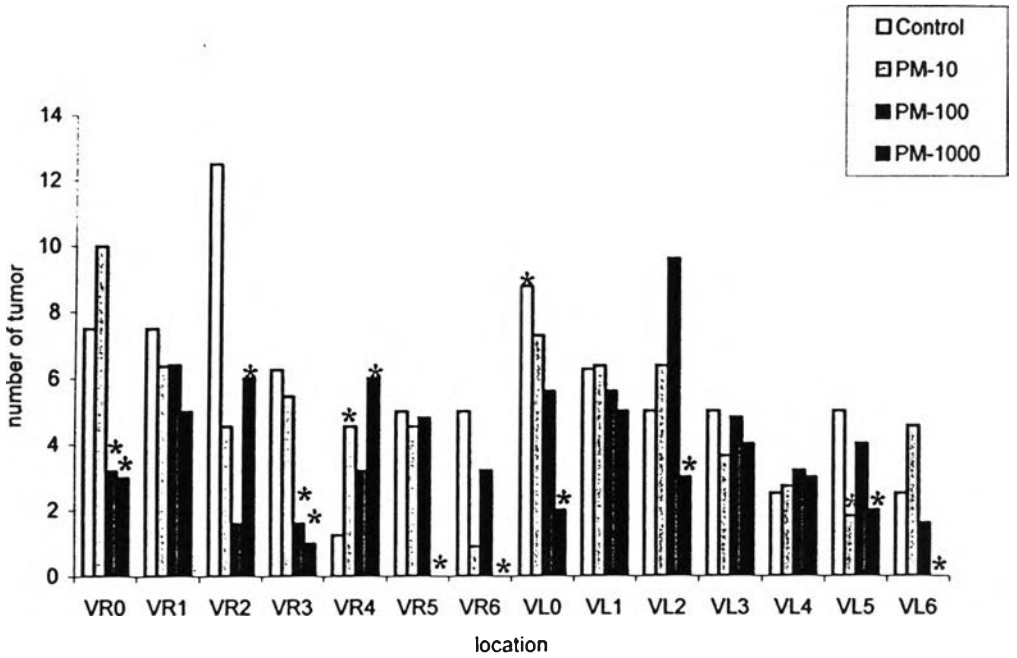


Figure 4.23 Distribution of tumor localization on preventive study of *P. mirifica* on DMBA induced mammary tumor rat at grossly day. The asterisk shows significant difference compared with control at the 0.05 levels.

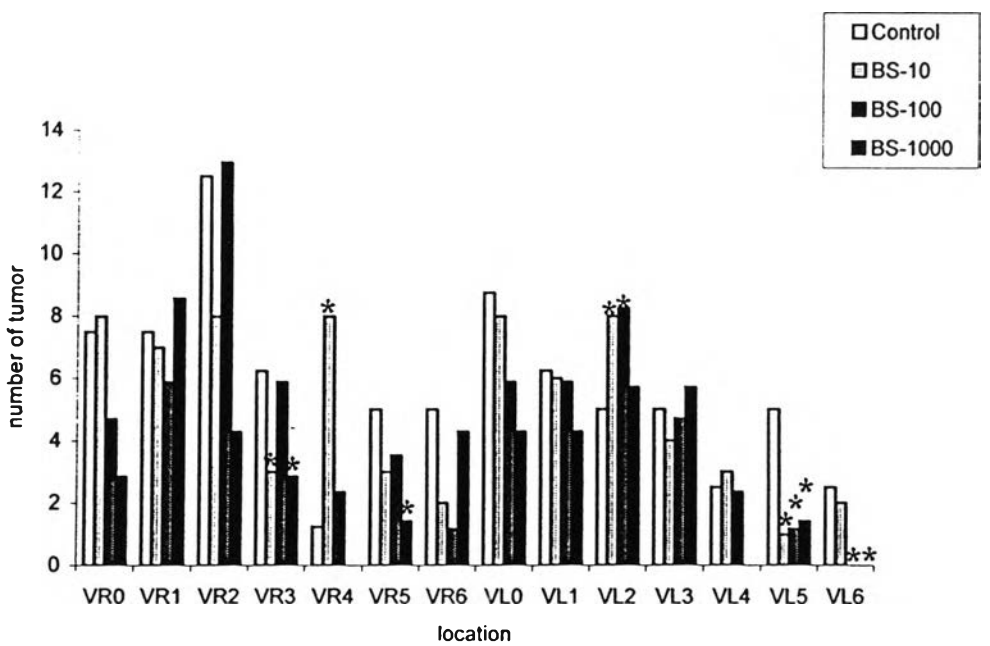


Figure 4.24 Distribution of tumor localization on preventive study of *B. superba* on DMBA induced mammary tumor rat at grossly day. The asterisk shows significant difference compared with control at the 0.05 levels.

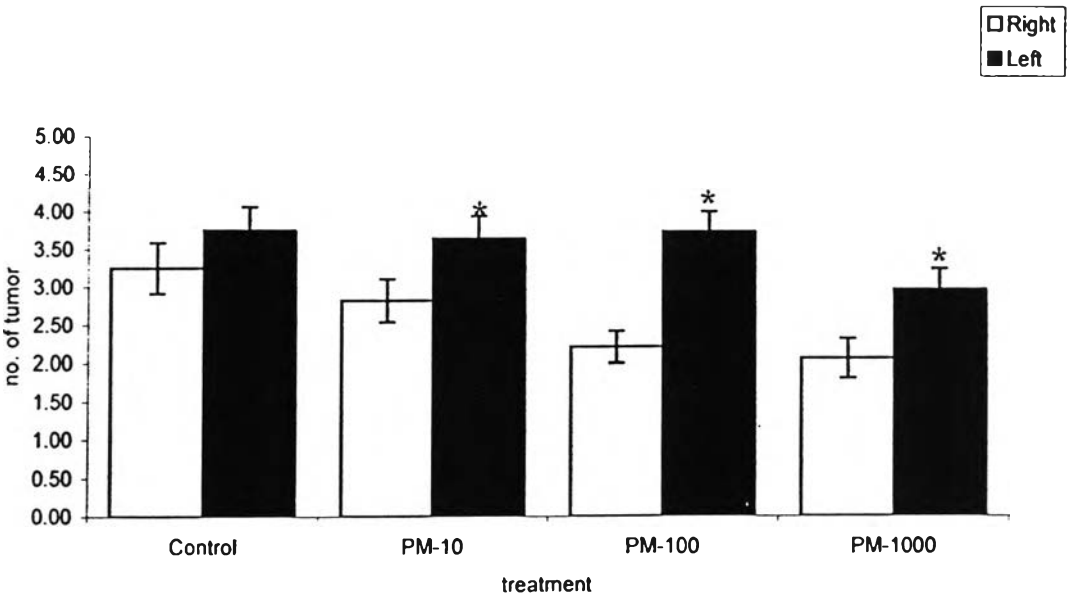


Figure 4.25 Mean ± S.E. value of distribution of tumor localization on preventive study of *P. mirifica* in DMBA induced mammary tumor rat at grossly day. The asterisk shows significant difference comparing within group at the 0.05 levels.

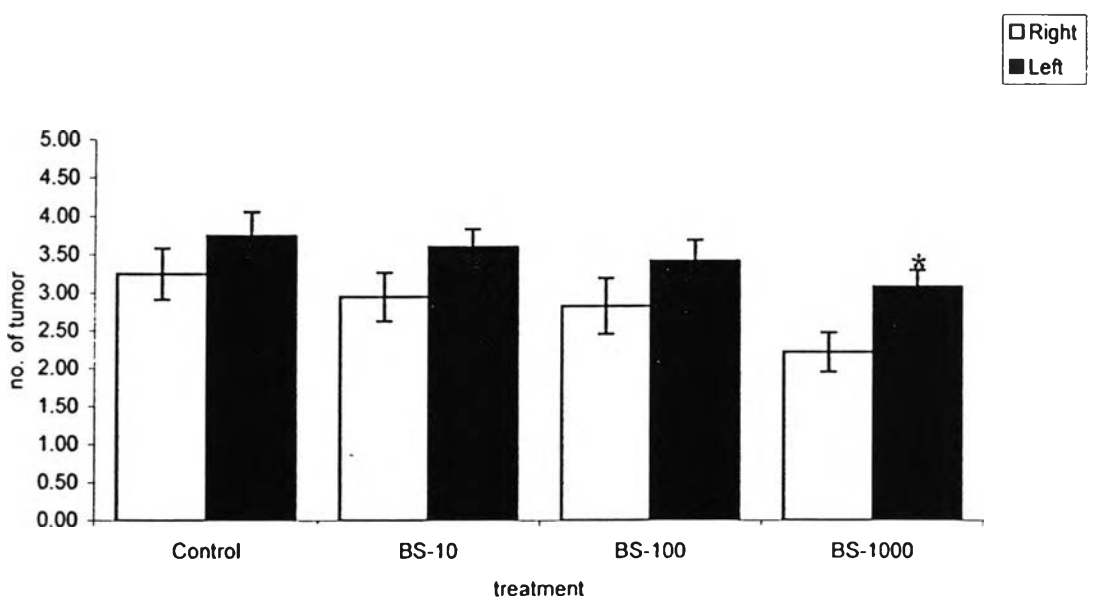


Figure 4.26 Mean ± S.E. value of distribution of tumor localization on preventive study of *B. superba* in DMBA induced mammary tumor rat at grossly day. The asterisk shows significant difference comparing within group at the 0.05 levels.

2) Tumor growth

Number of mammary tumor

The responses of tumor-developed rats to the feeding for 28 consecutive days with *P. mirifica* or *B. superba* and a single of carcinogen were palpated to detect for the onset of tumor. The number of developed tumor was recorded (Table 4.5). There was a significant difference between the multiplicities of tumor in various week of experiment. Mammary tumors of each group had been found between the forth to sixth weeks after treatment with DMBA (or the 8^{th} –11^{th} week of experiment). The numbers of tumor were increased and remain steady since the eleventh weeks. It is notice that the multiplicity of PM-1000 group was found significant lower than the control ($p<0.05$) since the first found until the end of experiment (Figure 4.27-4.28).

Table 4.5 Mean ± S.E. value of multiplicity of tumor on preventive study of *P. mirifica* and *B. superba* in DMBA-induced mammary tumor rats

Week	Control	PM-10	PM-100	PM-1000	BS-10	BS-100	BS-1000
9	0.4±0.3	0*	0*	0*	0.1±0.1*	0.1±0.1*	0.1±0.1*
10	0.3±0.2	0.1±0.1	0*	0.1±0.1	0.4±0.1	0.2±0.1	0.1±0.1
11	1.0±0.3	0.4±0.1*	0.2±0.1*	0.2±0.1*	0.7±0.2	0.8±0.3	0.3±0.1*
12	1.6±0.5	0.9±0.2	0.5±0.2*	0.3±0.2*	1.4±0.4	1.7±0.4	0.8±0.3
13	2.2±0.5	1.3±0.3	0.7±0.3*	0.4±0.3*	1.8±0.5	2.0±0.4	1.2±0.3
14	2.5±0.5	2.4±0.4	1.2±0.3*	0.8±0.3*	1.8±0.5	1.8±0.4	1.1±0.3
15	2.4±0.5	2.5±0.4	1.4±0.3	0.9±0.4*	2.1±0.5	2.0±0.4	1.2±0.3
16	3.1±0.6	2.5±0.4	1.6±0.3*	1.0±0.4*	1.8±0.4*	2.2±0.4*	1.6±0.2*
17	2.9±0.6	2.5±0.3	2.0±0.3	1.4±0.5*	1.8±0.4	2.5±0.4	1.9±0.3
18	3.0±0.4	2.9±0.4	2.2±0.3	1.4±0.4*	2.5±0.5	3.0±0.3	2.3±0.4
19	3.2±0.4	3.2±0.4	2.6±0.5	1.6±0.5*	3.0±0.4	2.9±0.4	2.6±0.4
20	3.2±0.4	3.1±0.4	2.8±0.3	1.5±0.5*	3.0±0.4	2.9±0.4	2.6±0.4

* = Statistical significant at $p<0.05$ compared with control

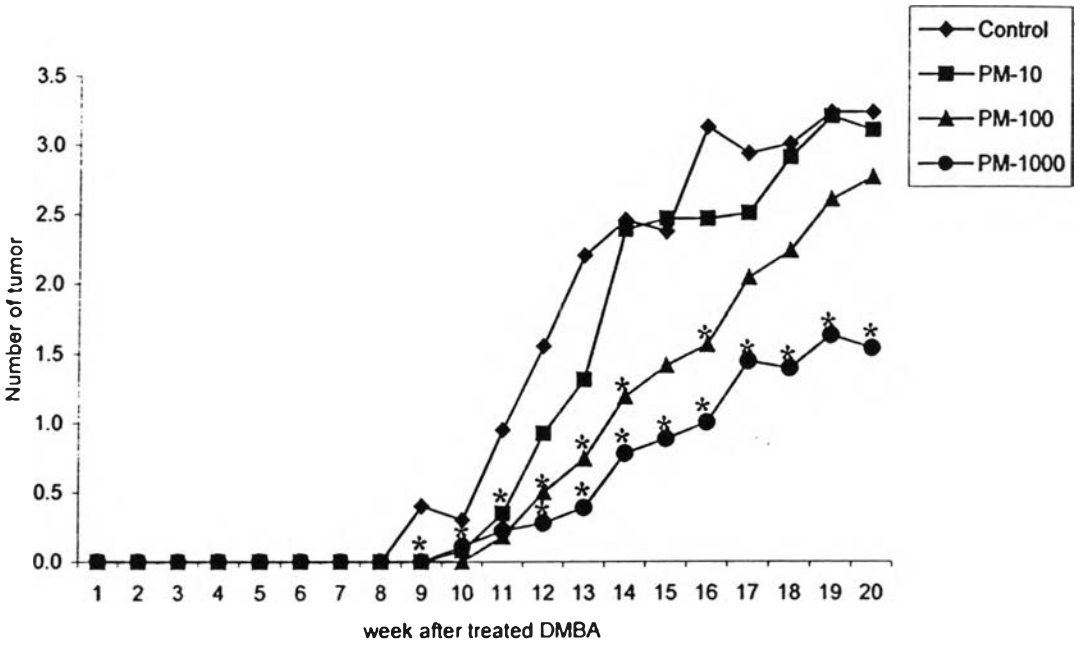


Figure 4.27 Multiplicity of tumor on preventive study of *P.* mirifica in DMBA-induced mammary tumor rats. The asterisk shows significant difference comparing with control at the 0.05 levels.

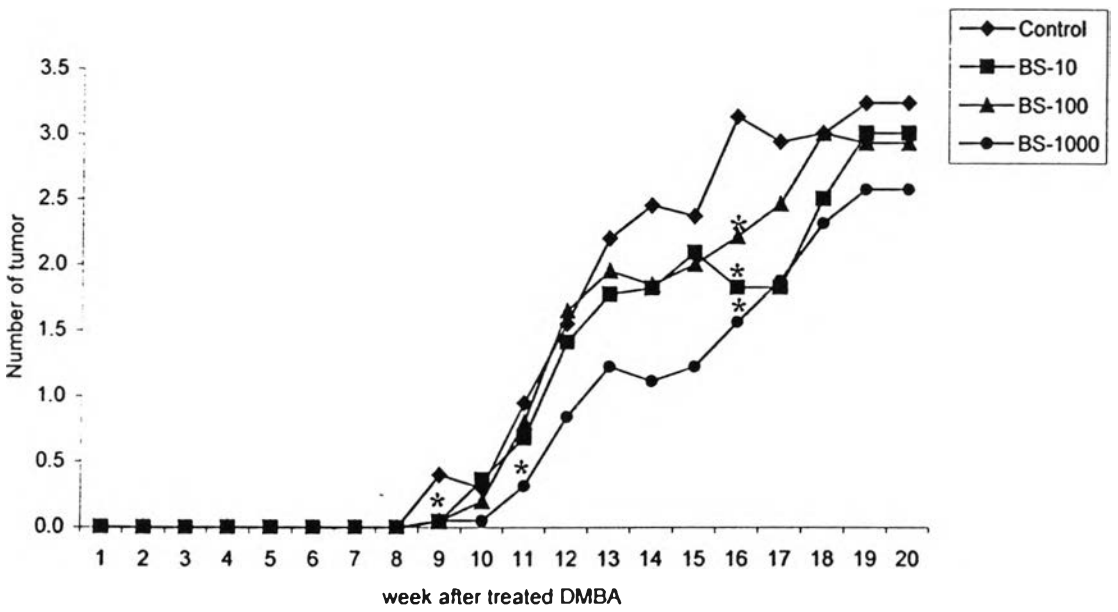


Figure 4.28 Multiplicity of tumor on preventive study of *B. superba* in DMBA-induced mammary tumor rats. The asterisk shows significant difference comparing with control at the 0.05 levels.

Weekly mean diameter of mammary tumor

The weekly mean diameter of mammary tumor of tumor-developed rats treated with 28 consecutive days of *P. minifica* and *B. superba* were recorded (Table 4.6 and Figure 4.29-4.30).

The mean diameter of mammary tumor in all groups was increased rapidly during the tenth to twentieth week of experiment. There was a statistical significant difference ($p<0.05$) including the control group in some weeks as follows.

In PM-10, the mean diameter of tumor was found significant lower at the 11^{th}-13^{th} weeks. In PM-100, there were significant lower at the 11^{th} – 18^{th} weeks of experiment. In PM-1000, there were significant lower at the 12^{th} – 13^{th}, 15^{th} –20^{th} week of experiment (Figure 4.29).

In *B. superba* treated group, there was a significant lower in BS-10 group at the 16^{th} – 20^{th} weeks and BS-1000 was found at the 16^{th} and 17^{th} weeks of experiment (Figure 4.30).

Table 4.6 Mean diameter of tumor on preventive study of *P. mirifica* and *B. superba* in DMBA-induced mammary tumor rats

Week	Control	PM-10	PM-100	PM-1000	BS-10	BS-100	BS-1000
9	0.01±0.01	0	0	0	0.01±0.01	0.01±0.01	0.01±0.01
10	0.06±0.02	0.01±0.01	0*	0.04±0.04	0.09±0.03	0.04±0.02	0.01±0.01
11	0.20±0.01	0.10±0.00*	0.13±0.01*	0.26±0.01	0.24±0.01	0.10±0.01	0.16±0.01
12	0.45±0.01	0.28±0.01*	0.18±0.01**	0.21±0.01*	0.45±0.01	0.55±0.01	0.34±0.01
13	0.69±0.01	0.46±0.01*	0.29±0.01**	0.34±0.11*	0.66±0.01	0.73±0.01	0.49±0.10
14	0.82±0.01	0.74±0.01	0.52±0.01*	0.61±0.13	0.76±0.01	0.80±0.01	0.53±0.11
15	0.98±0.01	0.90±0.01	0.64±0.01*	0.61±0.12*	0.98±0.10	0.97±0.12	0.66±0.12
16	1.15±0.01	1.02±0.01	0.77±0.01**	0.66±0.13**	0.71±0.01**	0.87±0.11	0.69±0.12**
17	1.27±0.01	1.09±0.01	0.98±0.01*	0.90±0.12*	0.72±0.01**	0.95±0.11	0.85±0.11*
18	1.37±0.01	1.27±0.01	1.08±0.01*	0.95±0.12**	0.95±0.01**	1.27±0.01	1.10±0.10
19	1.46±0.01	1.40±0.01	1.35±0.12	1.06±0.11*	1.12±0.01*	1.32±0.01	1.21±0.01
20	1.45±0.01	1.42±0.01	1.34±0.01	1.00±0.12**	1.14±0.01*	1.31±0.01	1.21±0.11

*, ** = Statistical significant at $p<0.05$ and 0.01 compared with control, respectively.

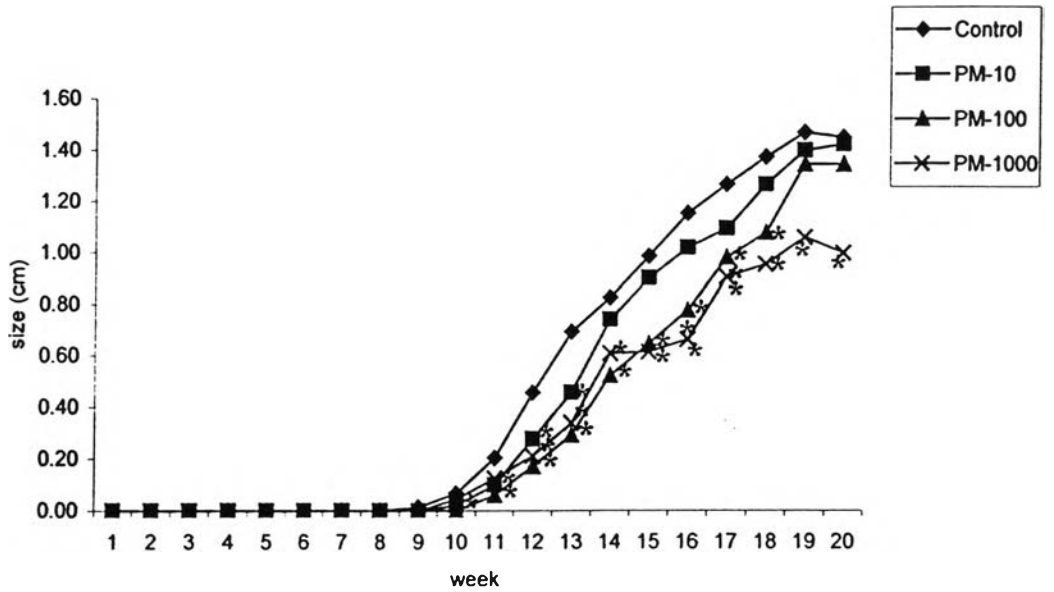


Figure 4.29 Mean diameter of tumor on preventive study of *P. mirifica* in DMBA-induced mammary tumor rats. The asterisk shows significant difference comparing with control at the 0.05 levels.

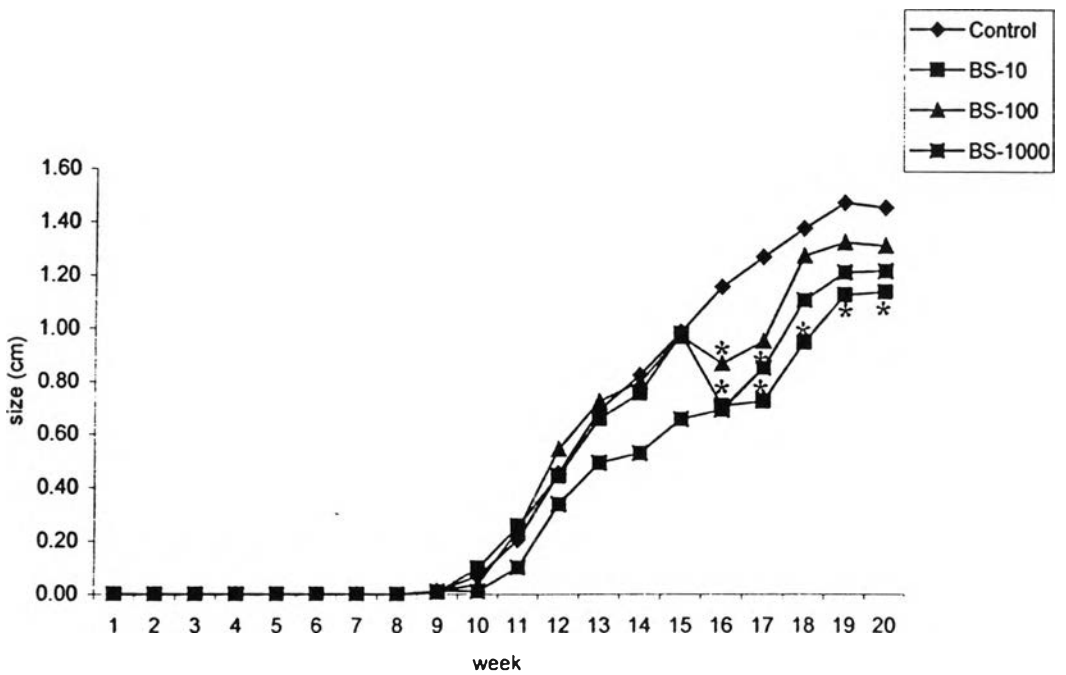


Figure 4.30 Mean diameter of tumor on preventive study of *B. superba* in DMBA-induced mammary tumor rats. The asterisk shows significant difference comparing with control at the 0.05 levels.

3) Tumor size at grossly day

At the end of experiment, the rats were sacrificed and tumors were evaluated for the multiplicity, weight and size (Figure 4.31 and Table 4.7). Mammary tumor was found as rolling up the subcutaneous nodules. The tumor surface was smooth and some lobulated and large blood vessels as shown in Figure 4.31. A red whorled appearance, necrosis and hemorrhage had occurred.

The multiplicity or number of tumor per rat at the right side of abdomen was lower significantly difference in PM-100, PM-1000 and BS-1000 group comparing with the control. However, total mean $\pm$ S.E. of multiplicity showed the significantly lower only in PM-1000 ($p<0.01$). The tumor weight, diameter and volume were statistical significantly lower than the control group in PM-1000 ($p<0.01$). Tumor diameter was significant higher in BS-10 ($p<0.05$) and BS-100 ($p<0.01$) group and tumor volume was found higher in BS2 group ($p<0.05$).

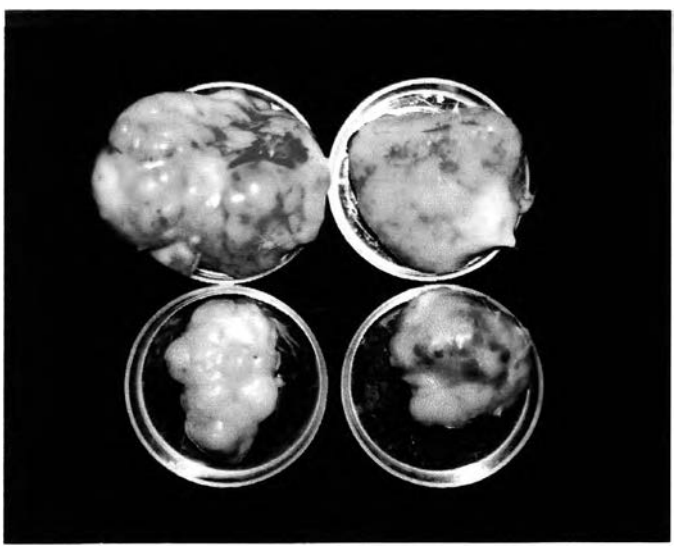


Figure 4.31 Mammary tumors were excised from female rat at the grossly day. The diameter of Petri disk is 3.5 cm.

Table 4.7 The preventive study of *P. minifica* and *B. superba* on DMBA-induced mammary tumor rats

Group	Multiplicity			Tumor weight (g)	Tumor Diameter	Tumor Volume (cm^3)
	Left	Right	Total mean			
Control	3.75±0.31	3.25±0.34	4.00±0.56	2.00±0.25	1.05±0.06	1.82±0.26
PM1	3.64±0.30	2.82±0.28	3.45±0.53	1.96±0.25	1.11±0.06	2.30±0.45
PM2	3.72±0.26	2.20±0.26*	2.92±0.38	2.17±0.26	1.16±0.07	1.96±0.62
PM3	2.95±0.28	2.05±0.28*	2.00±0.48**	1.13±0.22**	0.84±0.06**	0.86±0.22**
BS1	3.60±0.23	2.95±0.32	4.00±0.48	1.81±0.40	1.18±0.05*	2.06±0.29
BS2	3.41±0.27	2.82±0.37	3.24±0.61	1.99±0.26	1.34±0.11**	2.83±0.52*
BS3	3.07±0.22	2.21±0.26*	2.29±0.40**	1.89±0.37	1.04±0.08	1.52±0.32

*, ** = Statistical significant at $p<0.05$ and 0.01 compared with control, respectively.

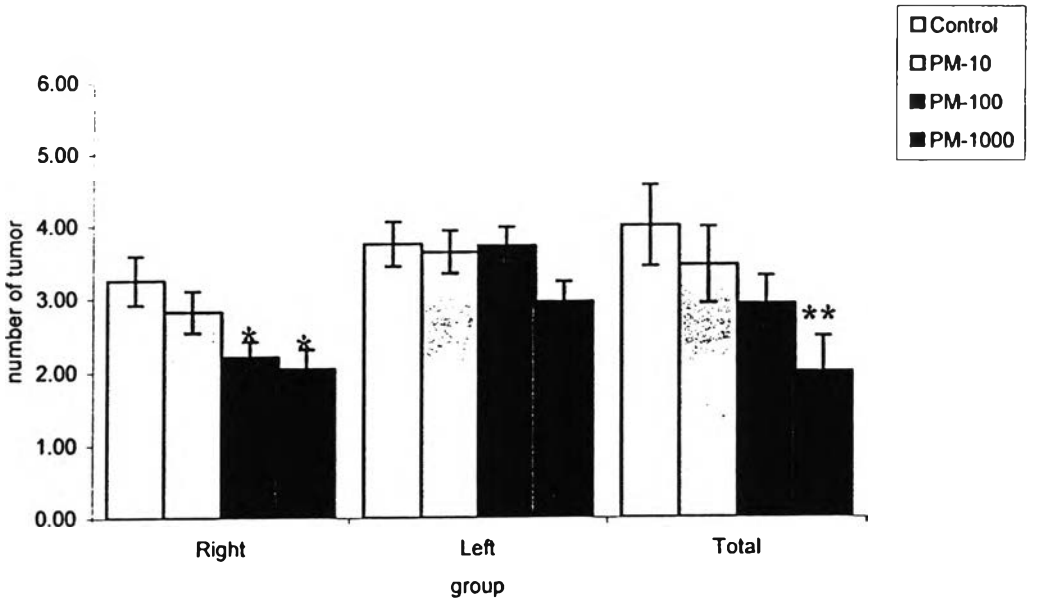


Figure 4.32 Multiplicity of tumor on preventive study of *P. mirifica* in DMBA-induced mammary tumor rats. One and double asterisk show significant difference comparing with control at the 0.05 and 0.01 levels, respectively.

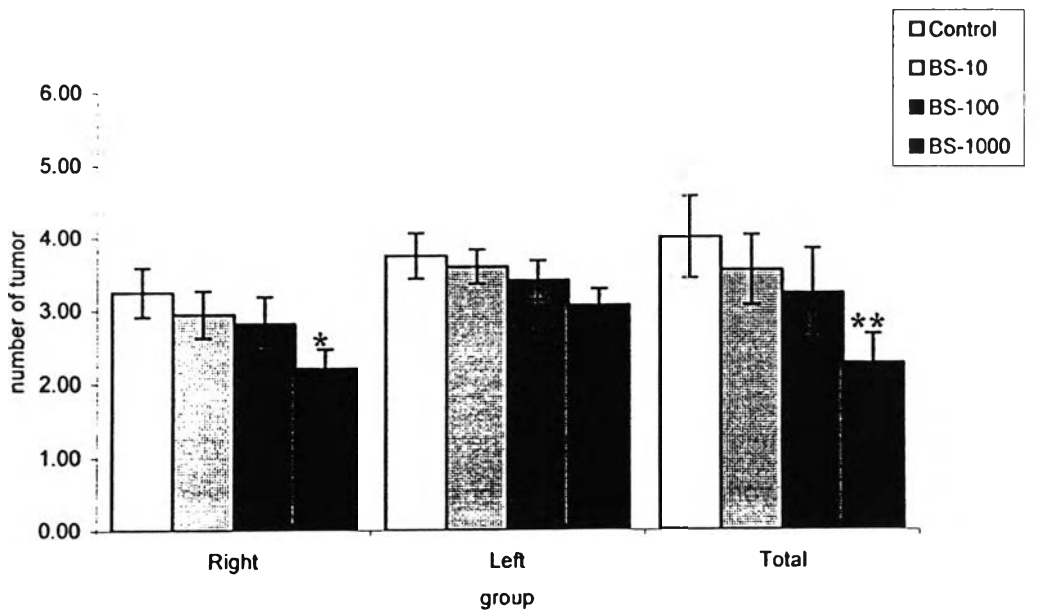


Figure 4.33 Multiplicity of tumor on preventive study of *B. superba* in DMBA-induced mammary tumor rats. One and double asterisk and cross show significant difference comparing with control at the 0.05 and 0.01 levels, respectively.

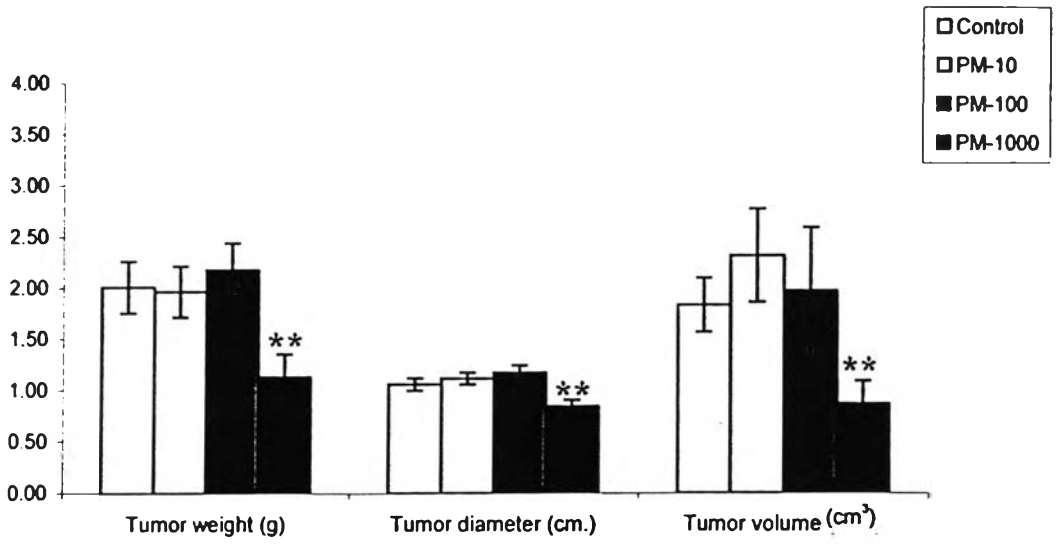


Figure 4.34 Weight, diameter and volume of tumor on preventive study in DMBA-induced mammary tumor rats. Double asterisks show significant difference comparing with control at the 0.05 and 0.01 levels, respectively.

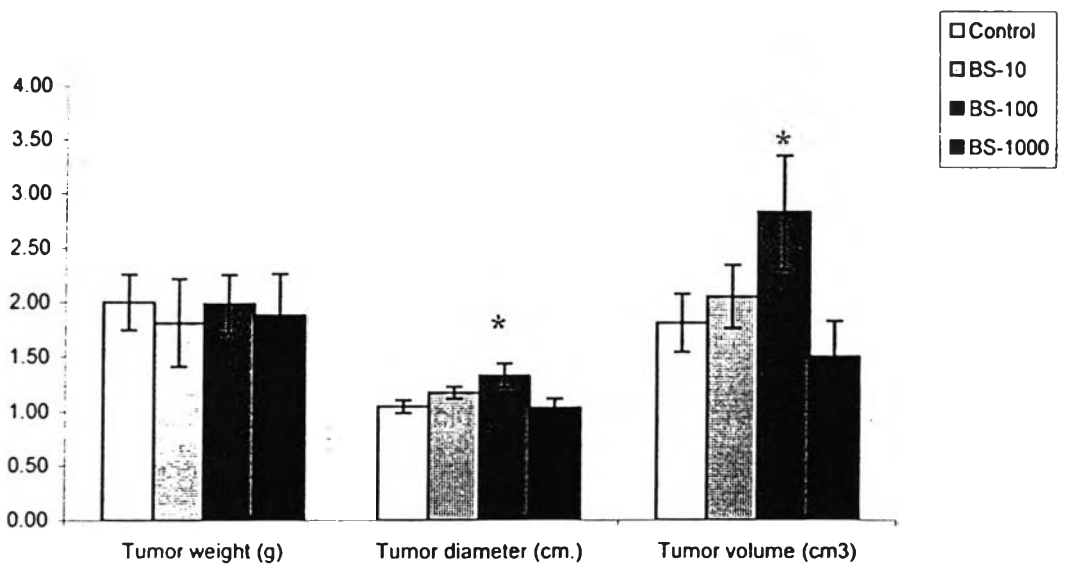


Figure 4.35 Weight, diameter and volume on preventive study of *B. superba* in DMBA-induced mammary tumor rats. The asterisk shows significant difference comparing with control at the 0.05 levels.

4) Histological study of mammary tumor

Histopathological examination of tumors was classified according to WHO (Young and Hallowes, 1973). All tumors was malignant which characterized into 5 types; adenocarcinoma, papillary carcinoma, anaplastic carcinoma, cribiform carcinoma and comedo carcinoma (Figure 4.36). Most of them were adenocarcinoma (Figure 4.36A), which showed in varied patterns. Acini showed vary in size and shape and lined by 1-2 layer of cuboidal epithelial, which was called as simple adenocarcinoma. More complexity of adenocarcinoma was also found. Papillary carcinoma was accompanied by an epithelial proliferation. The growth of loose and oedesmatous fibrovascular stroma and irregular spaces composing with the branching papillary fronds have occurred (Figure 4.36B). Anaplastic carcinoma was consisted with irregular sheets of epithelial cell arrangement and little stroma (Figure 4.36C). Cribiform carcinoma demonstrated the proliferation of epithelial cell in circumscribed and some secretion into acini can be occurred (Figure 4.36D). Some tumor was found alveolar adenocarcinoma with comedo pattern that central tissue necrosis is surrounded by glandular structures (Figure 4.36E).

Tumor was found as adenocarcinoma in all groups. The minority of tumor was papillary carcinoma. It is notice that the simple adenocarcinoma was demonstrated in the control group. No papillary carcinoma was observed in PM-1000, BS-100 and BS-1000. Comedo and analpastic carcinoma were rarely found. Furthermore, the mixing of tumor pattern can be found.

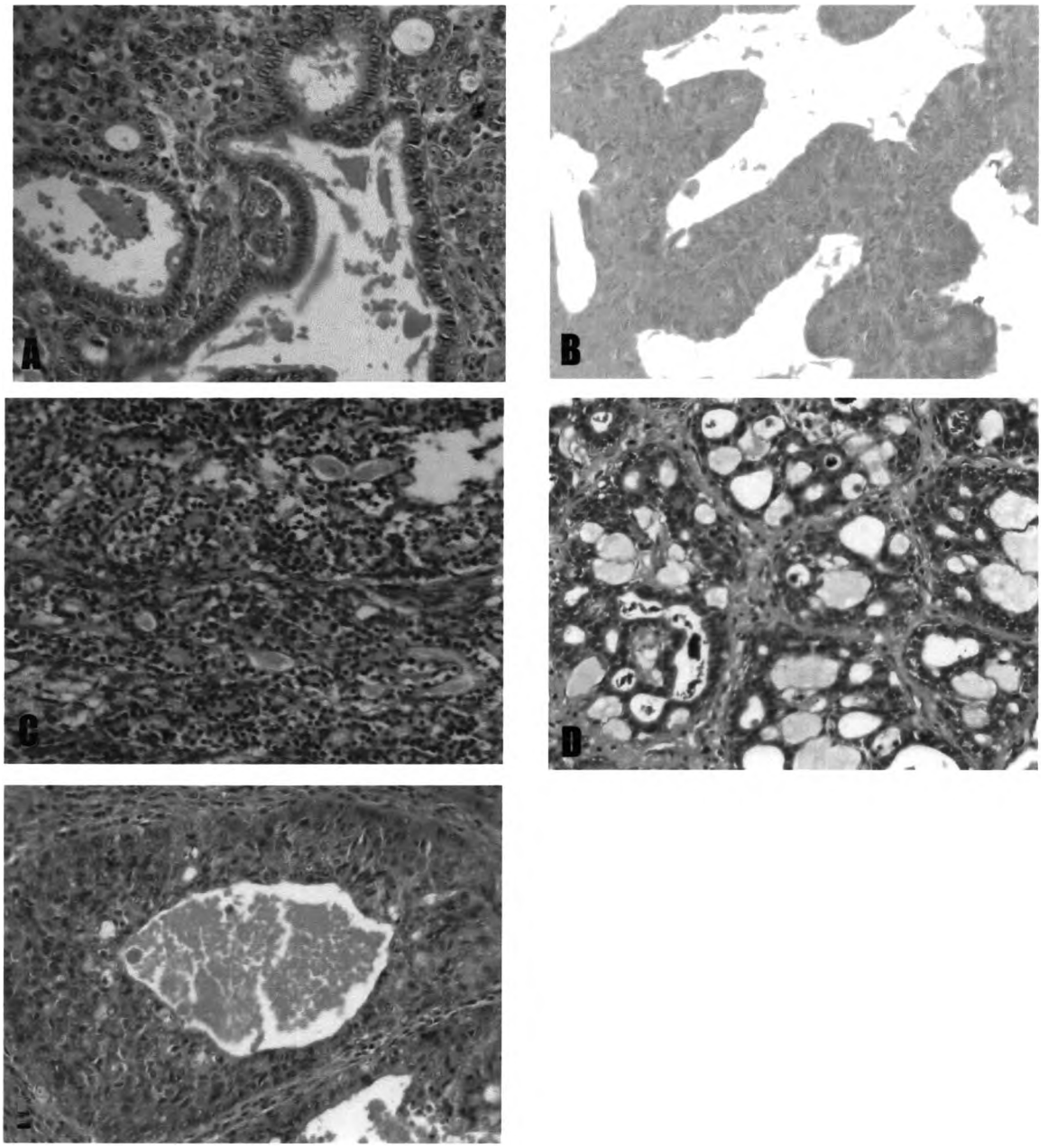


Figure 4.36 Histology of DMBA-induced mammary tumor in rat on preventive study of *P. mirifica* and *B. superba* followed the classification of WHO (Young and and Hallowes, 1973). Magnification x40.

A = Adenocarcinoma **B** = Papillary carcinoma

C = Anaplastic carcinoma **D** = Cribriform carcinoma

E = Comedocarcinoma

4.1.4 Estrogen receptor immunohistochemical in mammary tumor

Paraffin sections of mammary tumor were observed for ER-α and ER-β immunoreactivity. The evaluation of results was determined by a semiquantitative method. In both of ER-α and β was confined to the nuclei of all cell types. For the negative controls, no specific staining was observed as shown in Figure 4.37. Both receptors were found in mammary tumor compartment. However, there was difference in the staining intensity and localization. Epithelial compartment was found with weak staining intensity in all groups.

Their level of staining intensity as seen in Figure 4.38 and Table 4.8 shows all the semiquantitative results. Estrogen receptor was found as moderate staining intensity in both subtypes in all groups. A strong staining intensity could be observed with ER-α of BS-100 group. For the connective stroma, more positive stained than the layer of the surface epithelium.

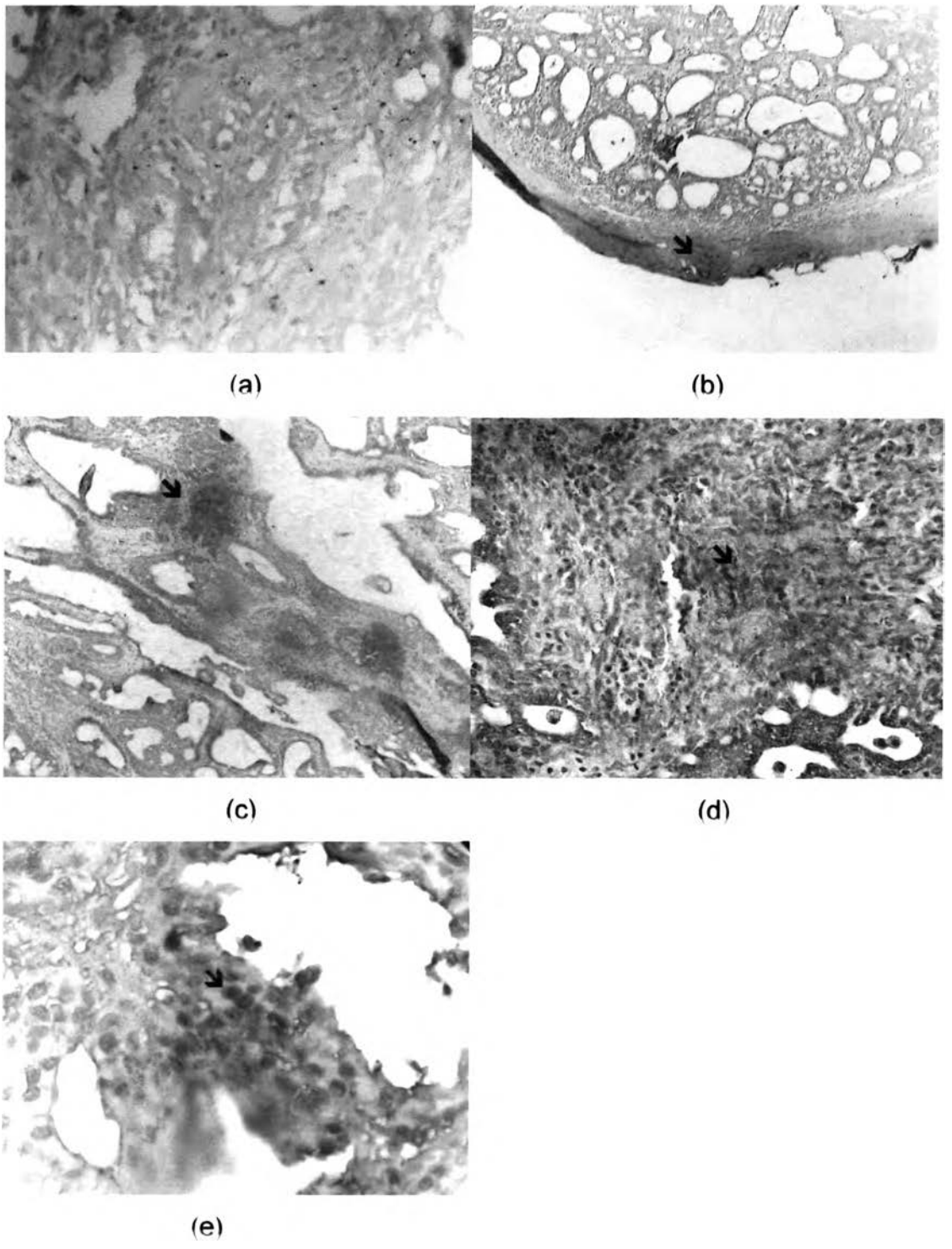


Figure 4.37 Representative immunohistochemical staining (↘) for ER-α and ER-β pattern of (a): Negative control (b): ER-α, (c-d): ER-β and (e): ER-β =x100

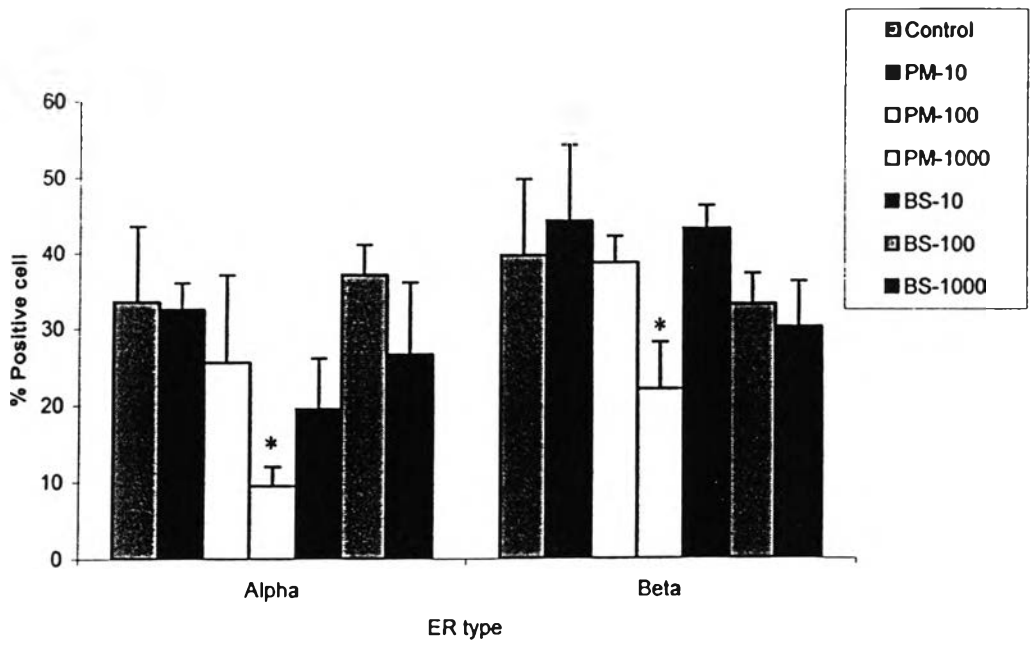


Figure 4.38 Percentage of positive cell staining of mammary tumor for estrogen receptor subtypes; alpha and beta immunostaining. The asterisk shows significant difference comparing with the control at the 0.05 levels.

Table 4.8 Percentage of positive cell staining of mammary tumor, for estrogen receptor subtypes; alpha and beta immunostaining

Group	Treatment	No. of rats	ER-α	ER-β	Ratio
1	Control	5	33.37	39.75	0.84
2	PM-10	5	32.06	44.42	0.72
3	PM-100	5	25.59	38.59	0.66
4	PM-1000	5	9.28*	22.05*	0.42*
5	BS-10	5	19.56	43.89	0.45
6	BS-100	5	37.12	33.57	1.11
7	BS-1000	5	26.42	30.66	0.86

*= significant difference comparing with the control at the 0.05 levels.

4.2 Anti-tumor activities of *P. mirifica* and *B. superba* in DMBA-induced rat mammary carcinoma

4.2.1 Growth rate and survival time

The growth rate of rats in the experiment groups is shown in Figure 4.39 and 4.40. The following data are details of each group.

In *P. mirifica* treated groups, the body weights were slightly increased as well as the control. The initial and final weights are shown in Table 4.9. There was significant difference in the body weight of PM-10 and PM-100 at the 3^{rd} weeks and PM-1000 at the 8^{th} week of experiment ($p<0.05$). The others were no significant difference.

In *B. superba* treated groups, the growth rate was higher than the control. In BS-10 group, the body weight was significant higher at the 4^{th} and $6^{th} - 8^{th}$ weeks of experiment. In BS-100 group, the body weight was significant ($p<0.05$) higher at the 4^{th}, $6^{th} - 7^{th}$, 15^{th} and $17^{th} - 18^{th}$ weeks of experiment. In BS-1000 group, the body weight was significant ($p<0.05$) higher at the 4^{th}, $13^{th} - 16^{th}$, and $19^{th} - 20^{th}$ week of the experiment ($p<0.05$). In addition, the body weight of the control and the *B. superba* group elevated inconsistency, with lower of the R^2 value (data not shown) when compare with the *P. mirifica*-treated group. The higher group of R^2 value was PM-10 and BS-1000, which were 0.8502 and 0.8202, respectively. The R^2 value of PM-10 and PM-1000 were 0.7073 and 0.7889, respectively. The control and BS-10 show the value of 0.6713 and 0.6890, respectively. The lowest value was 0.3573, which found in BS-10 group.

In the evaluation for survival, the animals were observed on the first day to the 20^{th} weeks of experiment. The following data are details of each group (Figure 4.41-4.42).

No death was found in all rats on the initial week to the 8^{th} weeks of experiment. In PM-1000 group, there was a significant decreasing than the control group in the 9^{th} weeks and 12^{th} week till the end of experiment ($p<0.05$). Others were not observed for difference ($p>0.05$). However, the mean survival times was not found significant different comparing with the control group ($p>0.05$) (Figure 4.43).

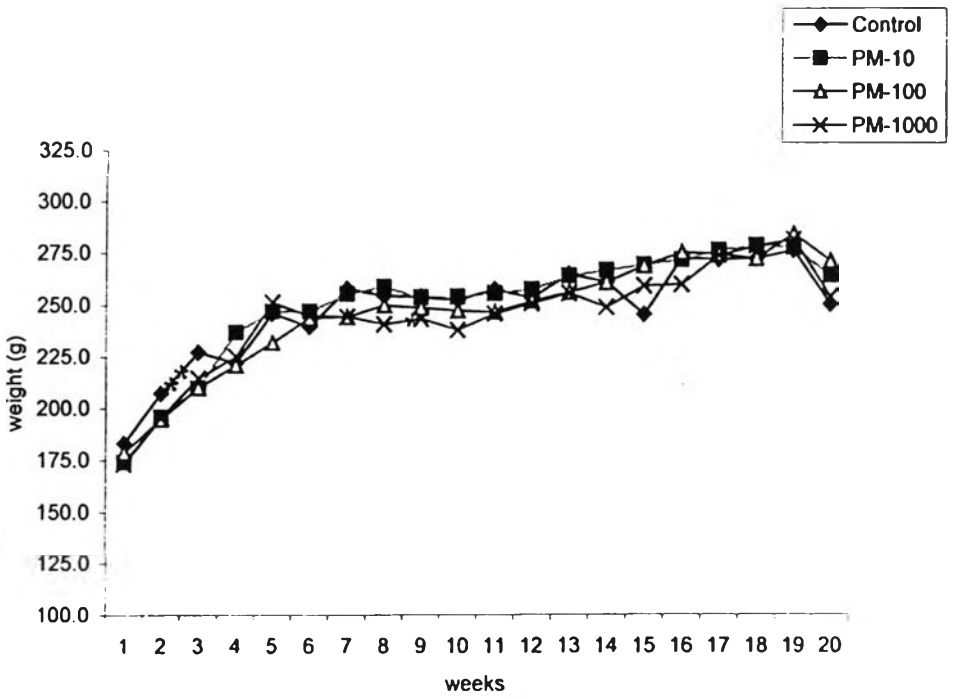


Figure 4.39 Body weight of DMBA-induced mammary tumor rats on anti-tumor study of *P. mirifica*. The asterisk shows significant difference comparing with control at the 0.05 levels.

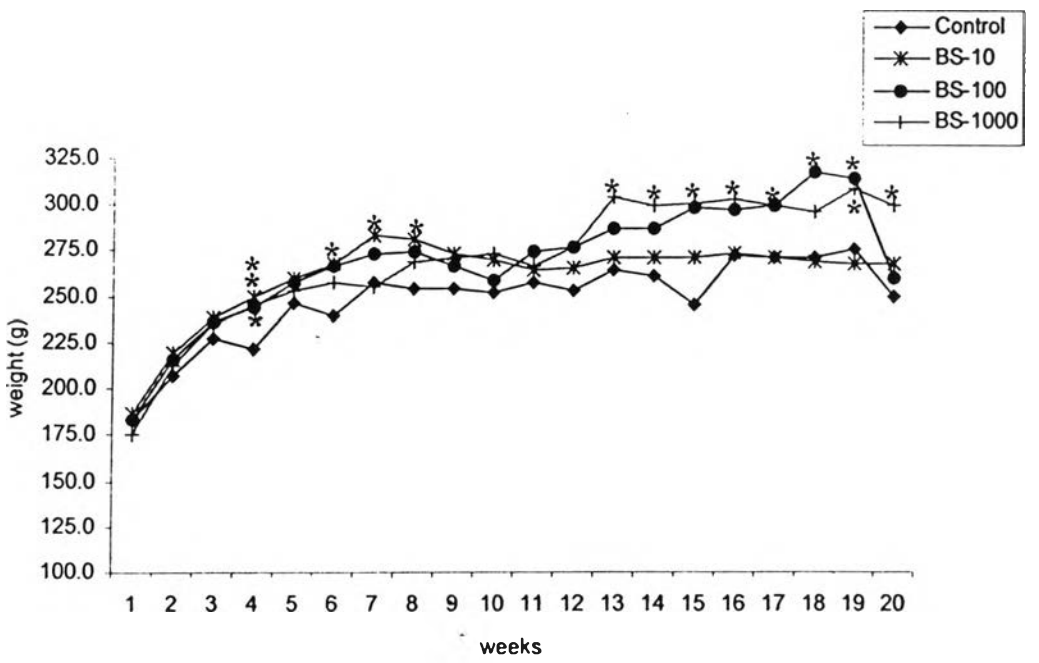


Figure 4.40 Body weight of DMBA-induced mammary tumor rats on anti-tumor study of *B. superba*. The asterisk shows significant difference comparing with control at the 0.05 levels.

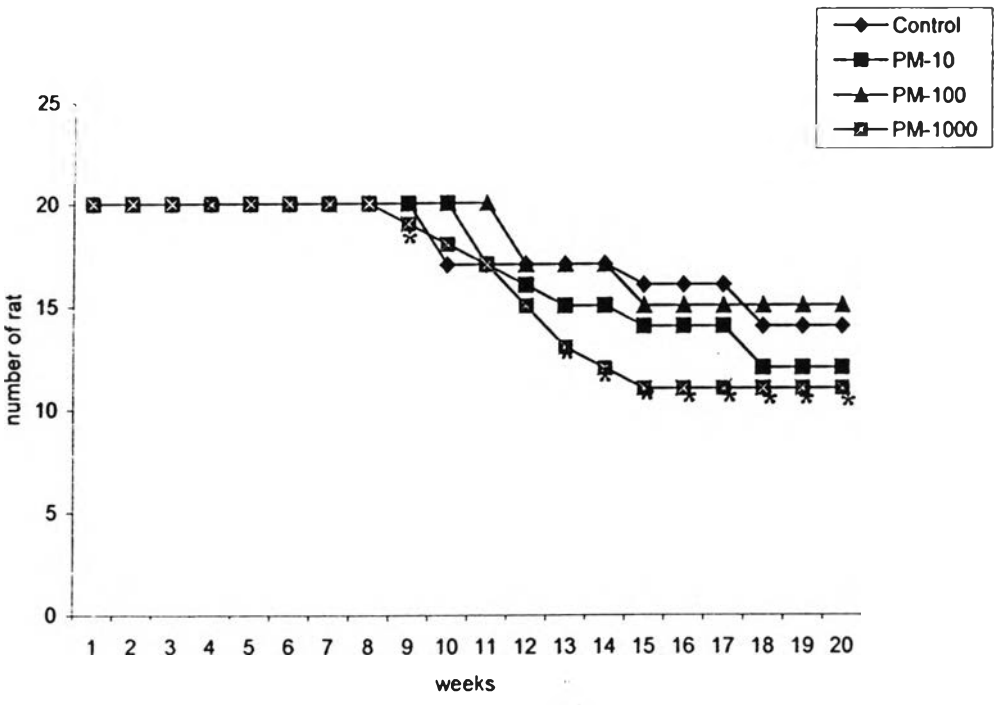


Figure 4.41 The survival rate of DMBA-induced mammary tumor rats on anti-tumor study of *P. mirifica*. The asterisk shows significant difference comparing with control at the 0.05 levels.

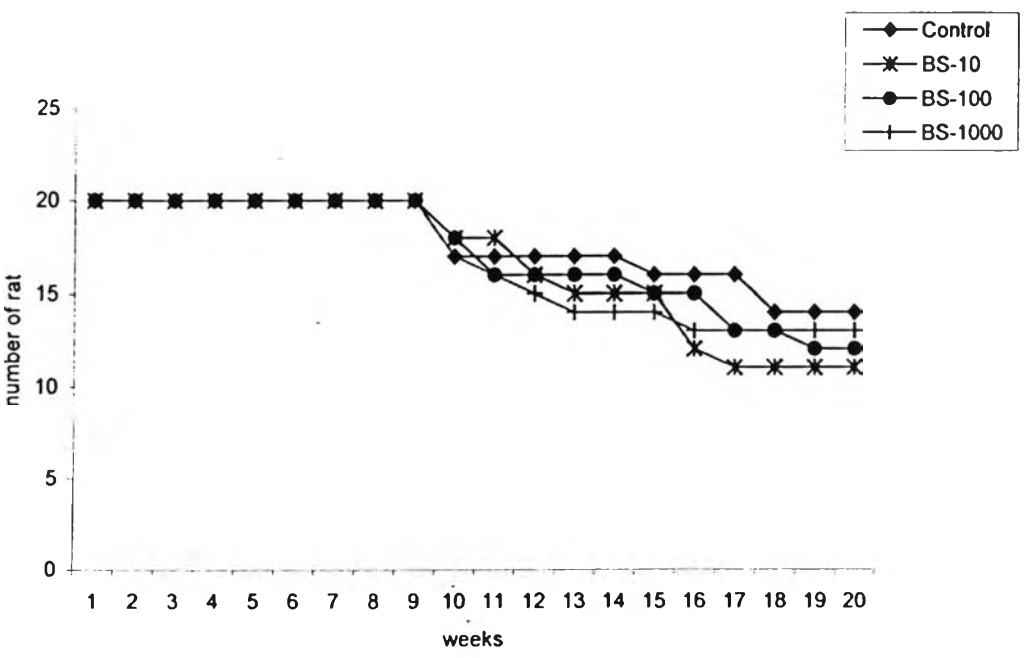


Figure 4.42 The survival rate of DMBA-induced mammary tumor rats on anti-tumor study of *B. superba*.

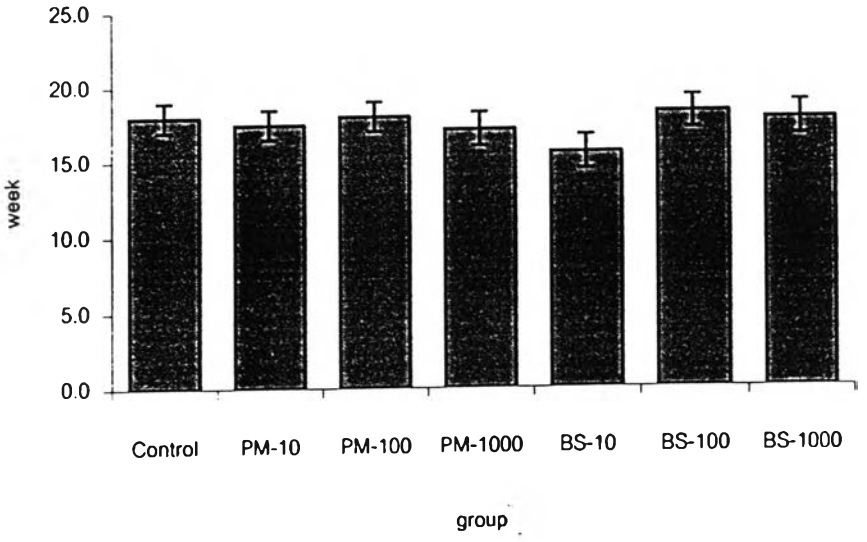


Figure 4.43 Mean survival time of DMBA-induced mammary tumor rats on anti-tumor study of *P. mirifica* and *B. superba*.

4.2.2 Organ

At necropsy day, the rats were weighed and sacrificed. Liver, ovary and uterus weight were recorded (Table 4.9)

The final body weight of the control was 249.8 ± 12.8 g (Mean ± S.E.). There was a significant difference in the BS-1000 group. For the weight gain comparison, all PM-treated group and BS-1000 were significant higher than the control (Figure 4.44-4.45).

1) Liver

The liver weight of DMBA-induced mammary tumor rats treated with *P. mirifica* and *B. superba* were recorded (Table 4.9). The highest mean ± S.E. value of the BS-1000 group was 10.049 ± 0.616 and the lowest mean ± S.E. was 7.869 ± 0.519 g of PM-1000 group. In *P. mirifica* treated group, the mean value was decreasing in dose dependent manner while it was not noted in *B. superba* treated group. Comparison of the liver weight with control was found to be no difference ($p>0.05$) (Figure 4.48-4.49).

The results of the histological examination of tissues taken from the rats treated with *P. mirifica* and *B. superba* at grossly day as shown in Figure 4.46 and 4.47 respectively. In the control group, the histological changes revealed mild fatty degeneration of the hepatic cells in the midzonal areas of liver (Figure 4.46A) and some hepatocytes were found clumping. The moderate fatty degeneration found in the group of PM-10 (Figure 4.46B), PM-100 (Figure 4.46C) and PM-1000 (Figure 4.46D). All of PM groups also found acidophilic cytoplasm in some of hepatocytes. The PM-10 group showed non-homogenous in straining. The PM-100 group showed an irregularity of the nuclear sizes. The large size of Kuffer's cell of PM-1000 group was also observed. In the BS-10 group (Figure 4.47B), the hepatocytes of portal triad were found dilatation. In the BS-100 group there was various degrees of histological changes as hydrophic swelling and fatty degeration in most of the midzonal area of the liver (Figure 4.47C). Furthermore, one animal in the BS-100 group showed massive necrosis as well as in BS-1000 group (Figure 4.47D).

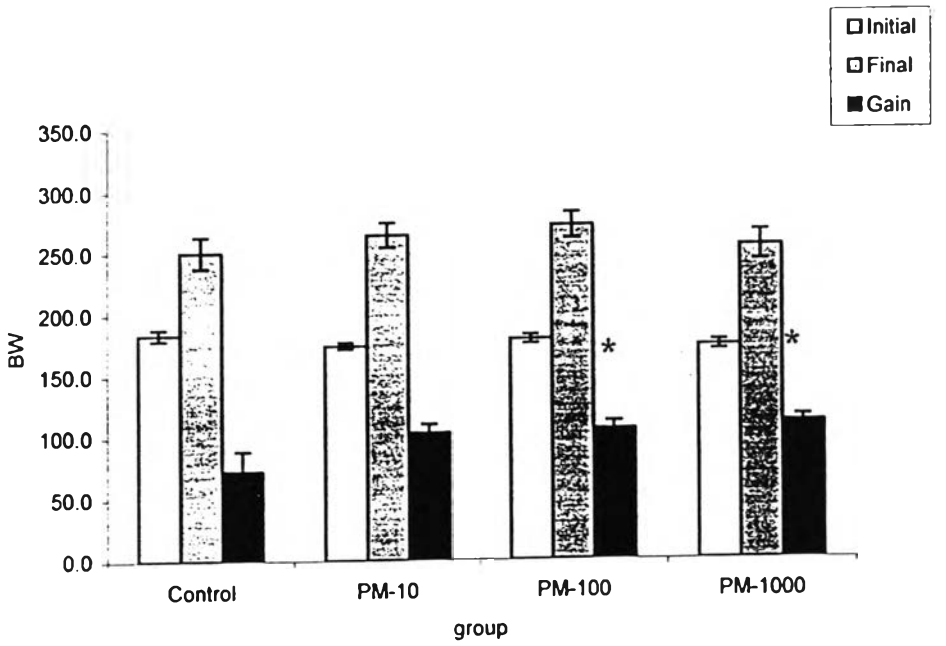


Figure 4.44 Mean ± S.E. of body weight of DMBA-induced mammary tumor in rats treated on anti-tumor study of *P. mirifica*. The asterisk shows significant difference comparing with control at the 0.05 levels.

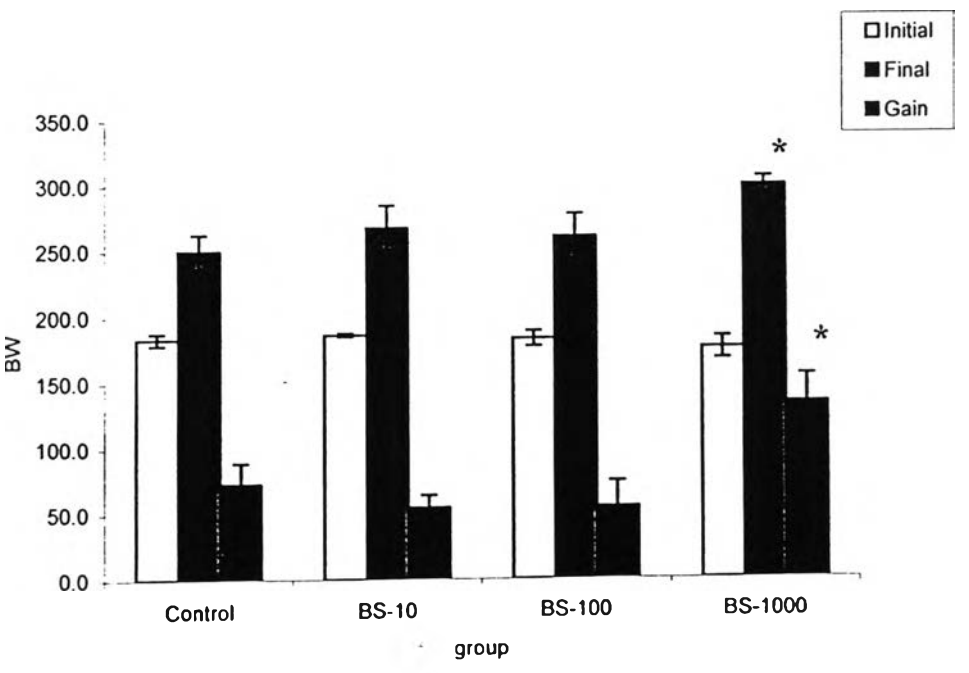


Figure 4.45 Mean ± S.E. of body weight of DMBA-induced mammary tumor in rats treated on anti-tumor study of *B. superba*. The asterisk shows significant difference comparing with control at the 0.05 levels.

Table 4.9 The final body and organ weight of DMBA-induced mammary tumor in rats treated with *P. mirifica* and *B. superba* (Mean ± S.E.)

	Control	PM-10	PM-100	PM-1000	BS-10	BS-100	BS-1000
Body weight (g.)							
Initial	183.1 ± 4.6	180.3 ± 2.8	178.7 ± 3.3	177.3 ± 3.8	186.3 ± 1.5	182.8 ± 5.8	175.5 ± 8.2
Final	275.6 ± 12.8	276.6 ± 9.9	286.1 ± 10.5	283.5 ± 11.9	264.6 ± 19.1	262.5 ± 15.9	298.8 ± 6.3*
Gain	92.7 ± 7.4	96.3 ± 4.6	107.4 ± 5.3*	106.2 ± 4.5*	94.5 ± 9.5	96.7 ± 19.5	134.0 ± 21.0*
Organ weight (g.)							
Liver	8.22 ± 0.55	8.71± 0.58	8.01± 0.37	7.87 ± 0.52	9.80 ± 1.32	8.19 ± 0.92	10.05 ± 0.62
Relative liver ($x10^{-2}$)	3.33 ± 0.02	3.30 ± 0.02	2.97 ± 0.01	3.12 ± 0.02	3.65 ± 0.03	3.20 ± 0.04	3.38 ± 0.02
Ovary	0.15 ± 0.01	0.15 ± 0.01	0.13 ± 0.01	0.14 ± 0.01	0.43 ± 0.19*	0.15 ± 0.01	0.33 ± 0.18
Relative ovary ($x10^{-3}$)	0.62 ± 0.08	0.59 ± 0.05	0.50 ± 0.05	0. 56 ± 0.04	1.56 ± 0.65*	0.60 ± 0.08	1.13 ± 0.61
Uterus	0.44 ± 0.05	0.38 ± 0.37	0.69 ± 0.12*	0.58 ± 0. 95	0.53 ± 0.17	0.60± 0.08	0.40 ± 0.03
Relative uterus ($x10^{-3}$)	1.74 ± 0.16	1.45 ± 0.12	2.53 ± 0.43*	2.26 ± 0.30	2.04 ± 0.62	2.25 ± 0.25	1.33 ± 0.07
Uterus horn length (c.m.)							
Uterus horn1	5.82 ± 0.11	5.88 ± 0.16	6.08 ± 0.14	5.76 ± 0.24	6.00 ± 0.21	6.20 ± 0.15	6.22 ± 0.20
Uterus horn2	5.66 ± 0.17	5.88 ± 0.13	5.91 ± 0.13	5.64 ± 0.14	5.98 ± 0.27	5.74 ± 0.17	6.24 ± 0.12*
Mean uterus horn	5.74 ± 0.11	5.88 ± 0.11	6.00 ± 0.11	5.71 ± 0.18	5.99 ± 0.18	5.97 ± 0.13	6.23 ± 0.12*

Relative organ = organ/BW, * = the mean difference is significant at the 0.05 compared with control.

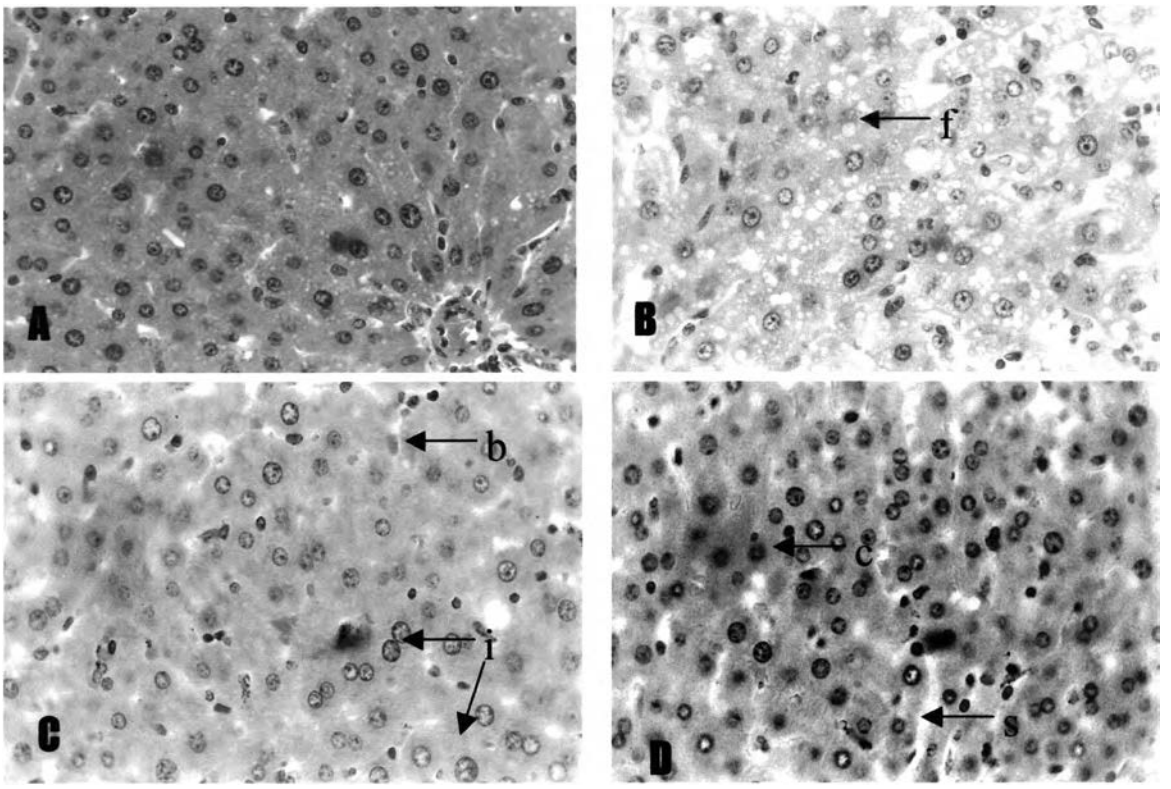


Figure 4.46 Liver tissues of DMBA-induced mammary tumor rats on anti-tumor study of *P. mirifica* (A) control, (B) PM-10, (C) PM-100 and (D) PM-1000. H&E straining for blood filtration (b), fatty degeneration (f), sinusoid dilation (s), irregularity of nuclear size (i) and clumping hepatocytes (c). Magnitude = x40.

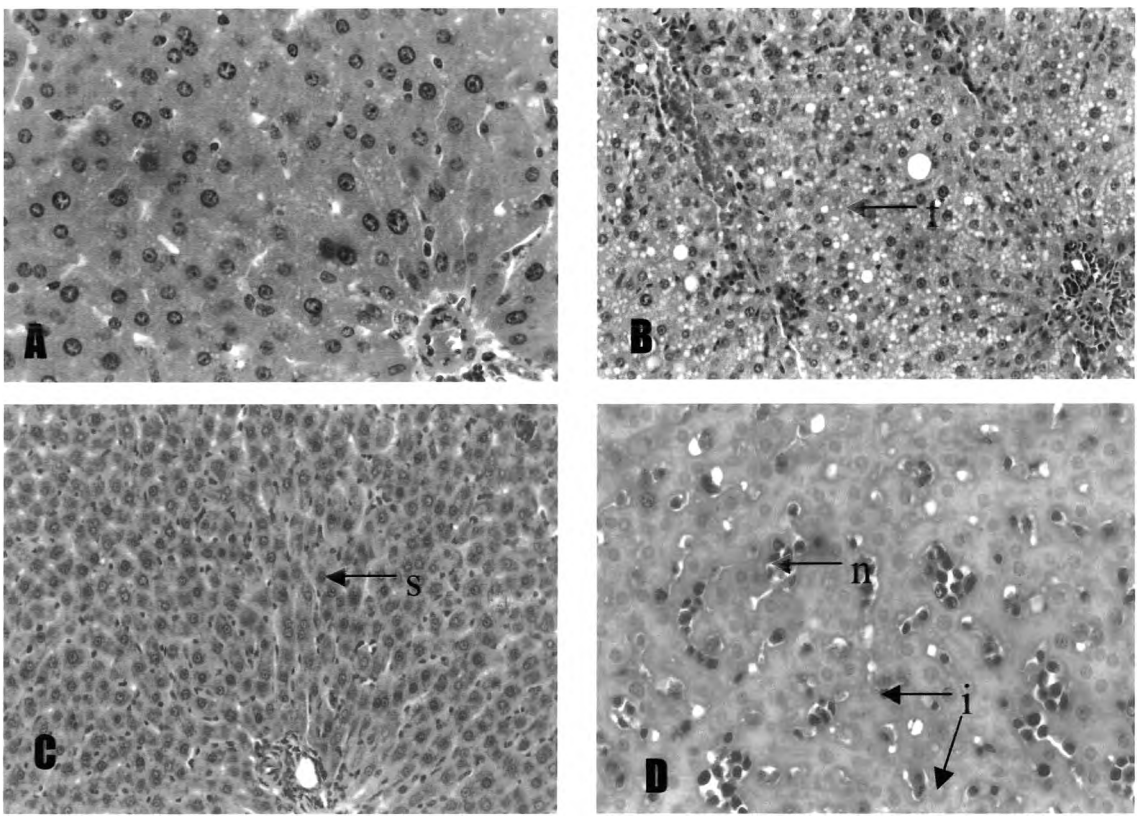


Figure 4.47 Liver tissues of DMBA-induced mammary tumor rats on anti-tumor study of *B. superba*. (A) control. (B) BS-10. (C) BS-100 and (D) BS-1000. H&E straining for fatty degeneration (f), sinusoid dilation (s), irregularity of nuclear size (i) and necrosis (n). Magnitude = x40.

2) Ovary

The weight of the ovary of DMBA-induced mammary tumor in rats treated with *P. mirifica* and *B. superba* were recorded (Table 4.9 and Figure 4.48-4.49). The highest mean ± S.E. value of ovary weight and relative ovary (ovary weight/BW) was 0.434 ± 0.194 and 0.156 ± 0.065 g, respectively in the BS-10 group. It is noticed that the mean value for liver of the BS-10 and BS-100 group was higher than the others. Comparison of the ovary and relative ovary weight with the control was found to be difference in BS-100 and BS-1000 ($p<0.05$).

The histology study of rat ovary in the control, *P. mirifica* and *B. superba* treated group showed the changing of follicles in various phases (Figure 4.50-4.51). In PM-10 group, blood infiltration containing several corpus lutea was found. Secondary follicles were also found. In PM-1000 growing follicles such as earcly primary follicle, late primary follicle and secondary follicle were dominantly shown. In PM-100 group, the numerous corpus lutea was seen. In *B. superba*-treated groups, Graafian follicle and corpus lutea were clearly found. In BS-100 group secondary follicles and blood infiltration in ovarian tissue were found.

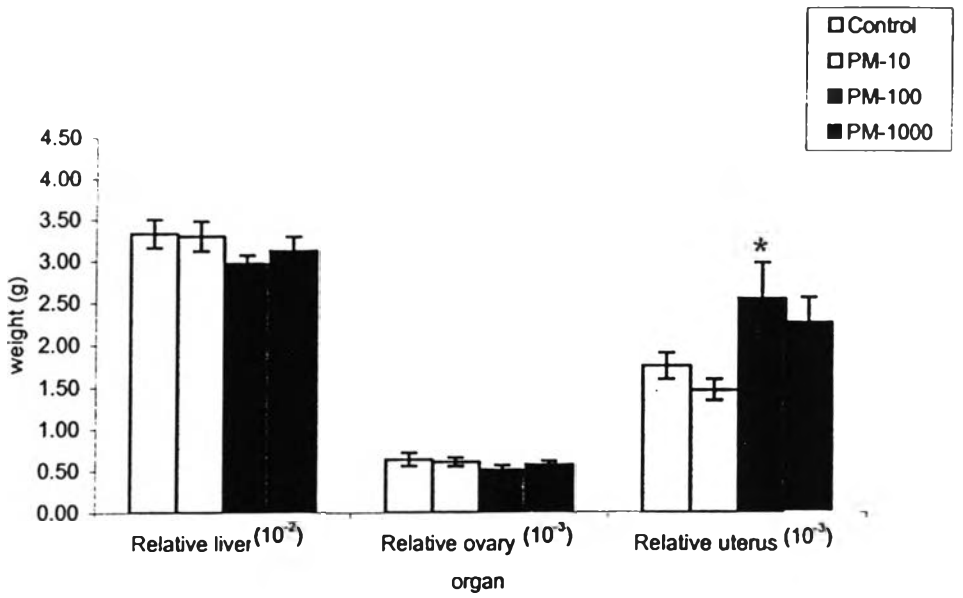


Figure 4.48 Mean ± S.E. of relative liver, ovary and uterus weight of DMBA-induced mammary tumor in rats on anti-tumor study of *P. minifica*. The asterisk shows significant difference comparing with control at the 0.05 levels.

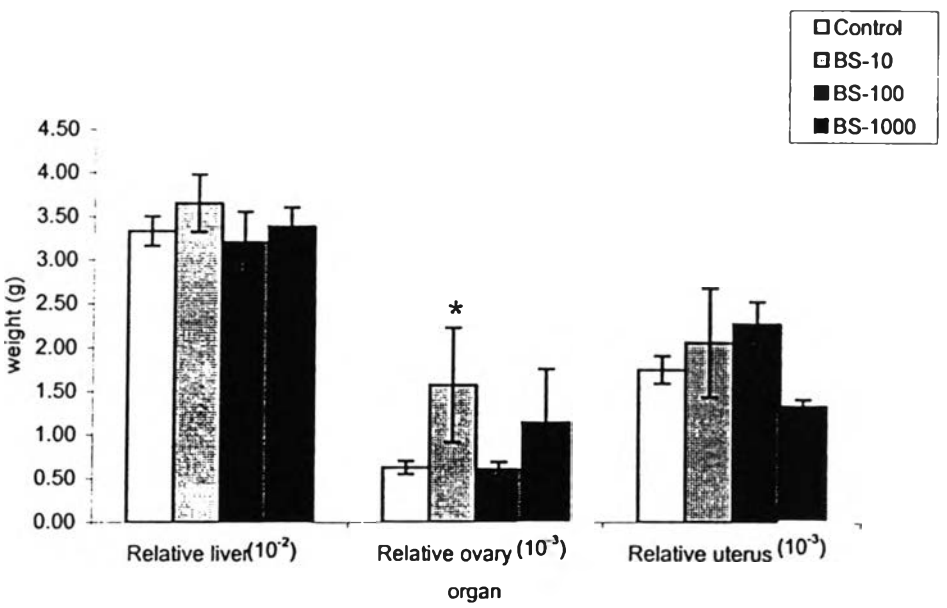


Figure 4.49 Mean ± S.E. of relative liver, ovary and uterus weight of DMBA-induced mammary tumor in rats on anti-tumor study of *B. superba*. The asterisk shows significant difference comparing with control at the 0.05 levels.

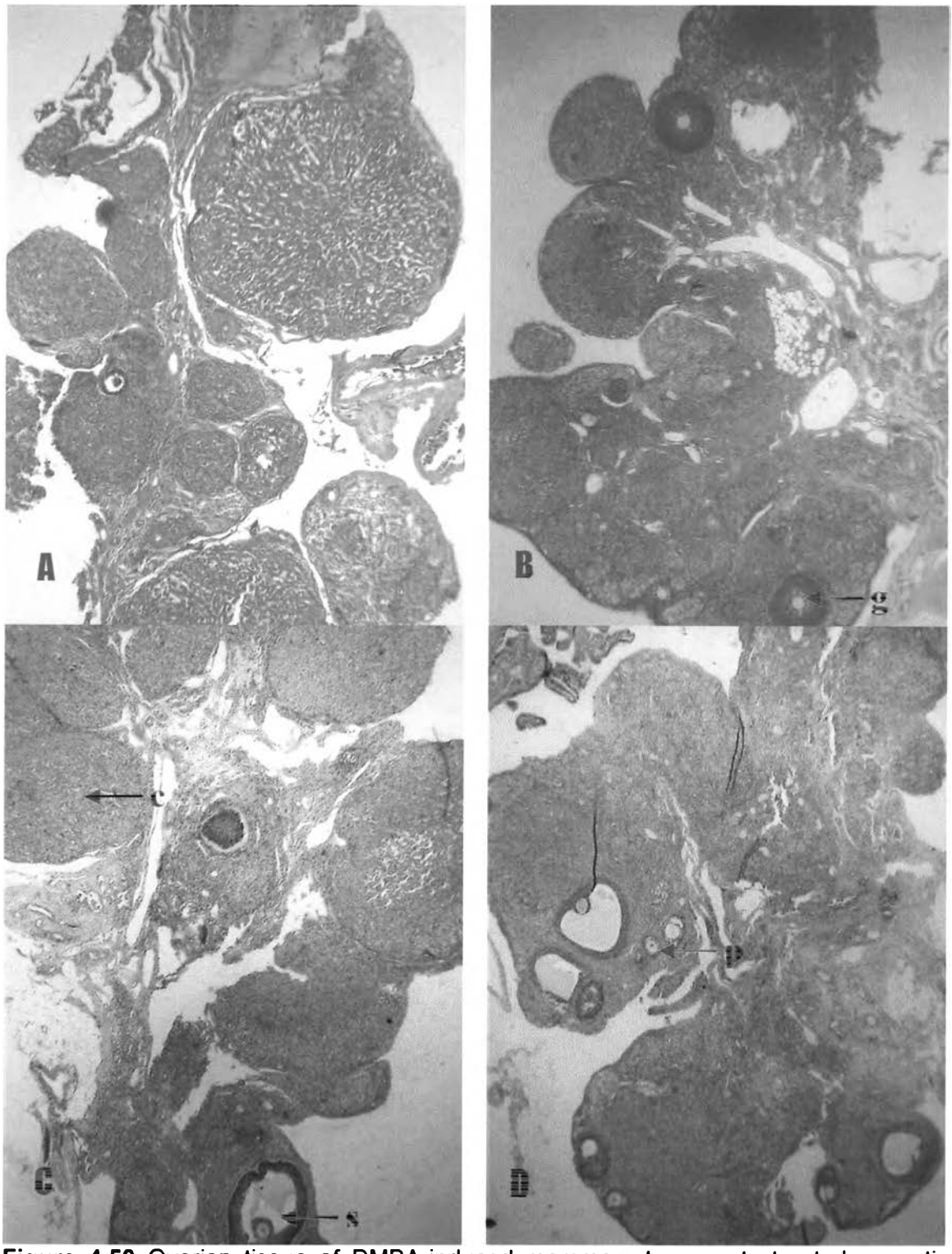


Figure 4.50 Ovarian tissue of DMBA-induced mammary tumor rats treated on anti-tumor study of *P. mirifca*. (A) Control group, (B) PM-10, (C) PM-100 and (D) PM-1000. H&E straining for primary follicle (p), secondary follicle (s), graafian follicle (g) and corpus luteum (c). Magnitude = x10.

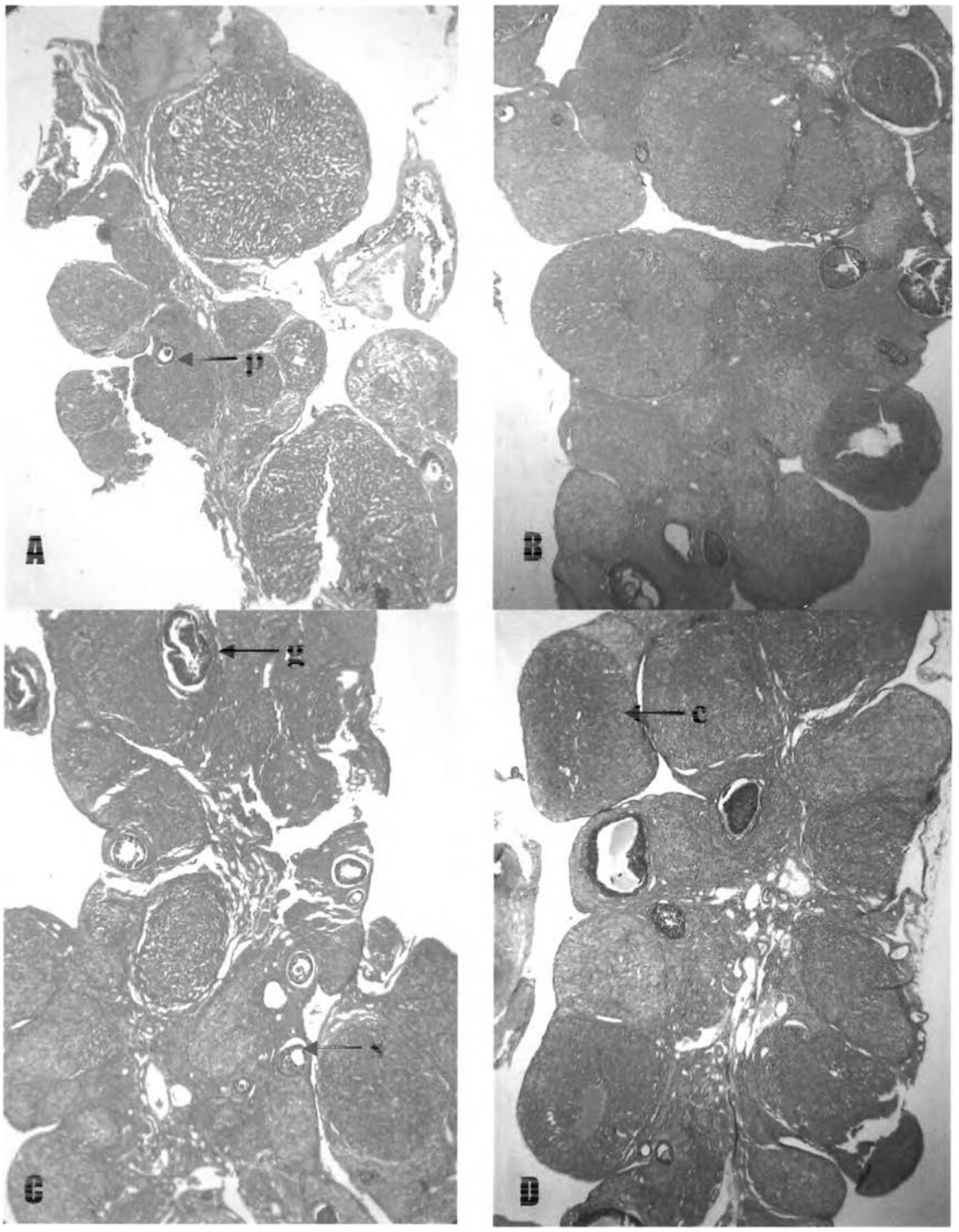
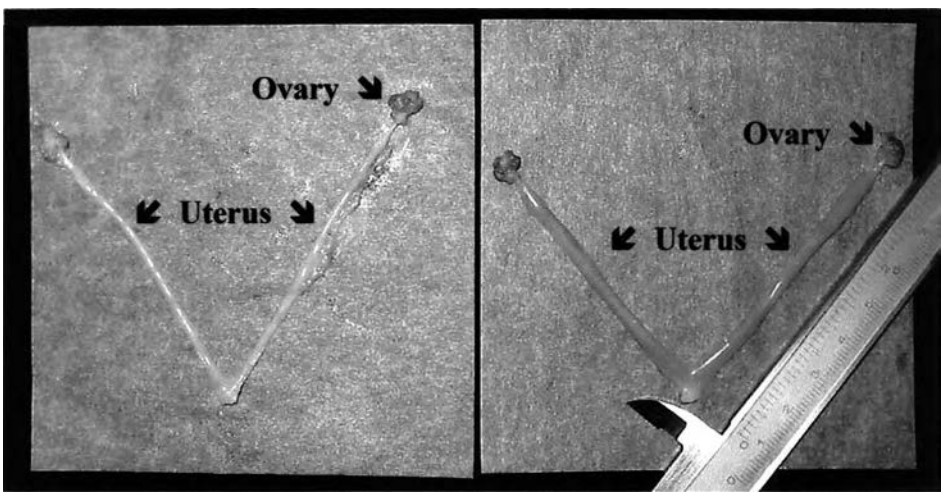


Figure 4.51 Ovarian tissue of DMBA-induced mammary tumor rats on anti-tumor study of *B. superba*. (A) Control group, (B) BS-10, (C) BS-100 and (D) BS-1000. H&E straining for primary follicle (p), secondary follicle (s), graafian follicle (g) and corpus luteum (c). Magnitude = x10.

3) Uterus

The weight of uterus and relative uterus of DMBA-induced mammary tumor in rat treated with *P. mirifica* and *B. superba* are recorded (Table 4.9 and Figure 4.48-4.49). The mean ± S.E. value of the PM-100 group was significant higher ($p<0.05$) than other group at 0.688 ± 0.116 g. and show statistical significant at $p<0.05$ level as compare with the control. In the PM-10 and BS-1000, the mean value of uterus weight was nearly closed while other groups show higher value in the same level. In highest weight of uterus appeared edema as shown in Figure 4.52. The length of uterus in BS-1000 group was higher significant than control group ($p<0.05$) (Figure 4.53-4.54).



(A) (B)

Figure 4.52 Ovary and uterus appearance of DMBA-induced mammary tumor rat (A) normal uterus and (B) edema uterus.

The histological study of *P. mirifica* treated group showed larger size of uterus than the control (Figure 4.55-4.56). In PM-10 and PM-1000, it is noticed that the mucosa epithelial cell lining had the cell lining in fully cuboidal shape and filled with mucous. In PM-10 group, the myometrium layer was thick. The uterine lumen in *P. mirifica* treated group showed a grooved curving. The endometrium stroma was found thicker and uterine gland was proliferated. *B. superba* treated group showed smaller size of uterus than control (Figure 4.56). The mucosa epithelial cell lining was the same as control; flattened cell lining and non-proliferated mucous in epithelial cells. The endometrium stroma was found the numerous uterine glands.

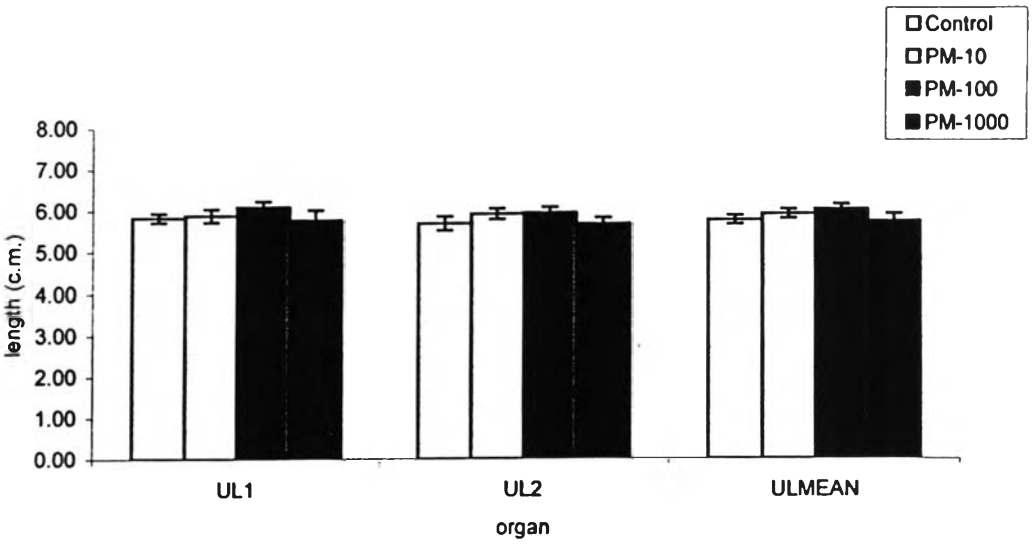


Figure 4.53 Mean ± S.E. of uterus horn length of DMBA-induced mammary tumor rats on anti-tumor study of *P. minifica*. The asterisk shows significant difference comparing with control at the 0.05 levels.

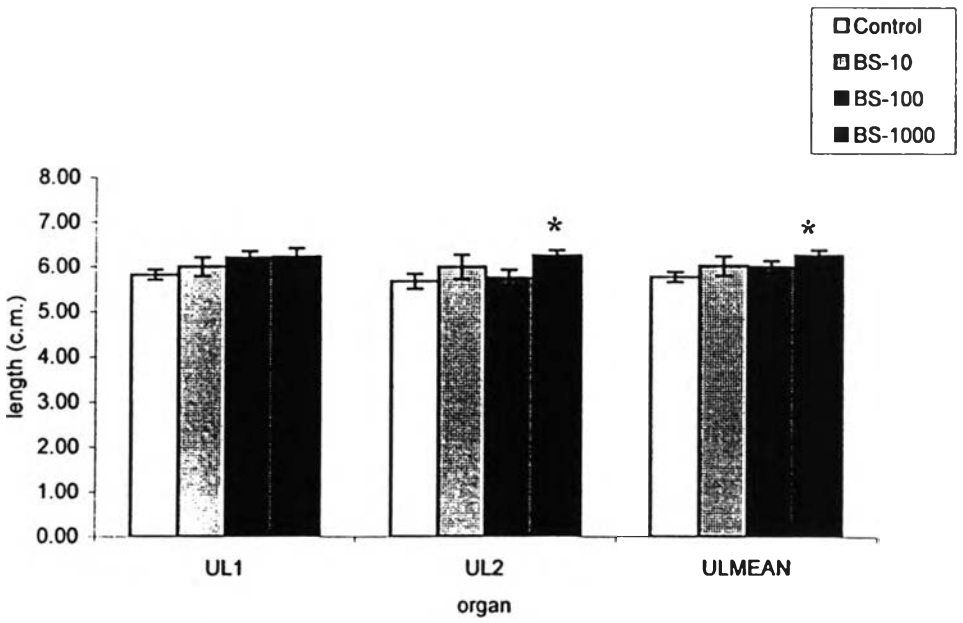


Figure 4.54 Mean ± S.E. of uterus horn length of DMBA-induced mammary tumor rats on anti-tumor study of *B. superba*. The asterisk shows significant difference comparing with control at the 0.05 levels.

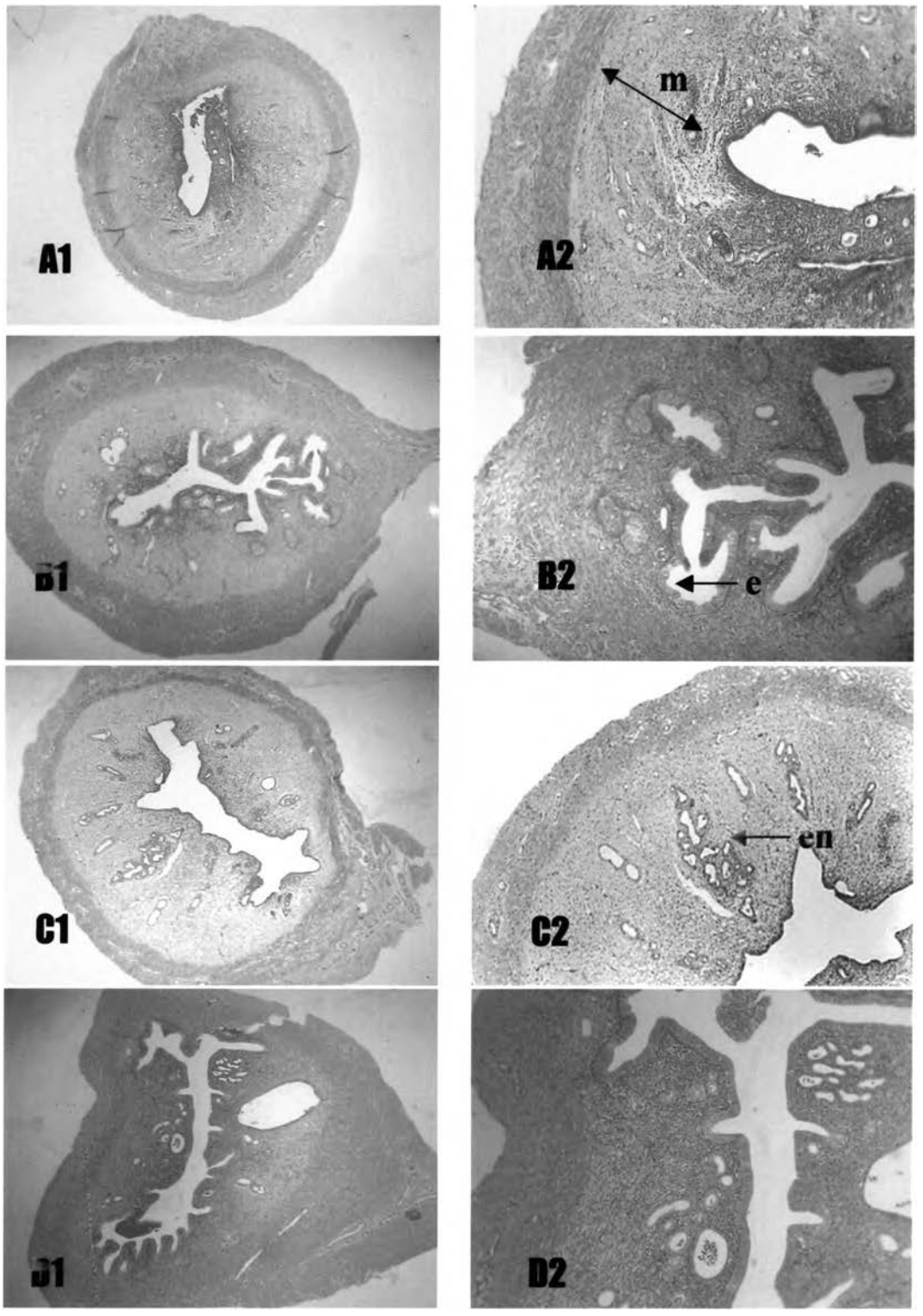


Figure 4.55 Uterus tissues of DMBA-induced mammary tumor rats on anti-tumor study of *P. mirifica*. (A1-2) control group. (B1-2) PM-10. (C1-2) PM-100 and (D1-2) PM-1000. H&E straining for mucosa epithelial (e), endometrium gland (en) and myometrium layer (m). Magnitude of A1, B1, C1, D1 = x10 and A2, B2, C2, 21 = x40.

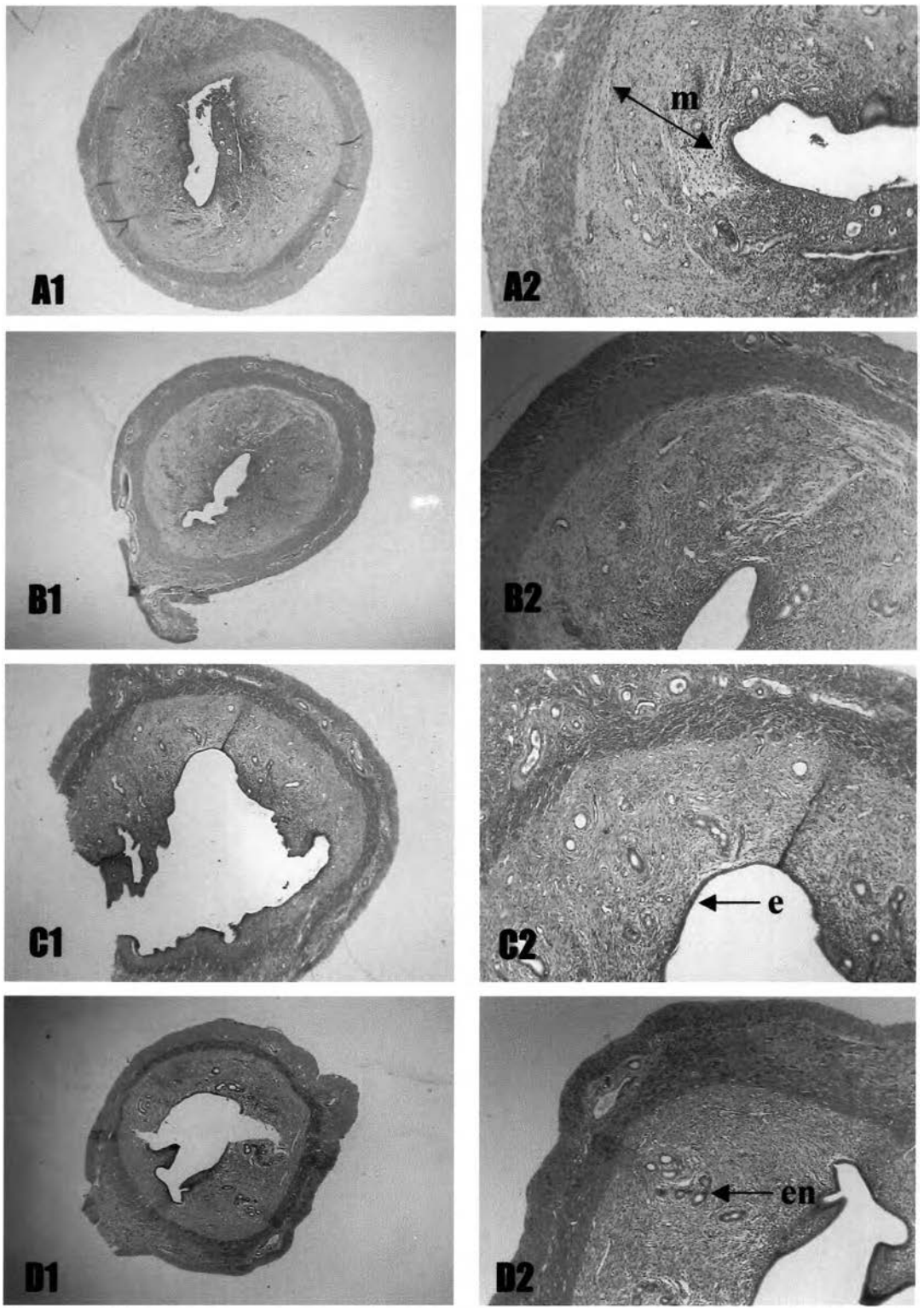


Figure 4.56 Uterus tissues of DMBA-induced mammary tumor rats on anti-tumor study of *B. superba*. (A1-2) control group, (B1-2) PM-10, (C1-2) PM-100 and (D1-2) PM-1000. H&E straining for mucosa epithelial (e), endometrium gland (en) and myometrium layer (m). Magnitude of A1, B1, C1, D1 = x10 and A2, B2, C2, 21 = x40.

4.2.3 Effect of *P. mirifica* and *B. superba* on tumorigenesis

1) Tumor induction and anti-tumor activity evaluation

After a single feeding of 80 mg/kg BW of 7,12 DMBA at the 50-day-old ages of eight SD rats. All rats, which developed tumor, were divided into a control and three of treated groups (20 rats each) using by location and occurrence time of tumors.

All rats were survived and were found for tumor incidence between 4-10 weeks as shown in Table 4.10. Total mean ± S.E. was 6.35 ± 0.11 weeks. The time of initial tumor occurrences were no significant differences (p = 0.562).

Table 4.10 Time of the first tumor occurrences on DMBA-induced mammary tumor in rats

Group	Week			
	Mean	**Std. Error**	**Minimum**	**Maximum**
Control	6.25	0.25	4.00	8.00
PM-10	6.00	0.24	5.00	8.00
PM-100	6.27	0.25	4.00	8.00
PM-1000	6.69	0.24	6.00	8.00
BS-10	6.27	0.23	5.00	8.00
BS-100	6.39	0.40	4.00	9.00
BS-1000	6.80	0.49	5.00	10.00
Total	6.35	0.11	4.00	10.00

The location of tumor firstly occurrences on DMBA-induced mammary tumor in rats were divided similarly with no significant difference (p = 0.682)(data is not shown).

At the grossly day, the tumors were found distributed in all mammary gland position, which defined into 16 position (Table 4.11). The pattern of tumor occurrence in both side showed in Figure 4.57-4.58. The over all tumor at grossly day (n= 20) are shown in Table 4.10. The maximum and minimum were 118 and 65 for BS-10 and PM-1000 group, respectively. Descending the overall tumor are BS-10 (118) > Control = PM-10 (100) > PM-100 (99) > BS-100 (97) > BS-1000 (90) > PM-1000 (65).

Considering in each position, VL2 position has got high score in PM-10 (19), control (16), BS-10 (15), PM-100 (14) while as PM-1000, BS-100 and BS-1000 got the same score of 13 tumor per 20 rats. VL6 position has got zero in control and PM-1000

group. Furthermore, in PM-1000 showed zero score in VL2 and VL6. Comparing the mean and S.E. of tumor number distributed in both side with control group, there was significant difference in some location as shown in Table 4.11.

Table 4.11 Mammary tumor localization on anti-tumor study of *P. mirifcia* and *B. superba* in DMBA-induced rat

No.	Location	Control	PM-10	PM-100	PM-1000	BS-10	BS-100	BS-1000
1	VR0	4	11	13*	4	13*	7	5
2	VR1	9	1*	9	9	8	8	8
3	VR2	10	11	7	10	13	3*	5
4	VR3	10	4	7	10	8	5	13
5	VR4	7	7	3	7	15	10	8
6	VR5	4	4	4	4	5	8	8
7	VR6	0	6	4	0	5	3	3
10	VL0	10	9	9	10	8	7	8
11	VL1	7	7	9	7	5	7	5
12	VL2	16	19	14	16	10	8	3
13	VL3	4	7	9	4	13*	13*	13*
14	VL4	11	7	4*	11	5	10	5
15	VL5	4	3	3	4	5	3	5
16	VL6	3	3	4	3	8	3	5
Total		100	100	99	65	118	97	90

* = Statistical significant at $p<0.05$ compared with control.

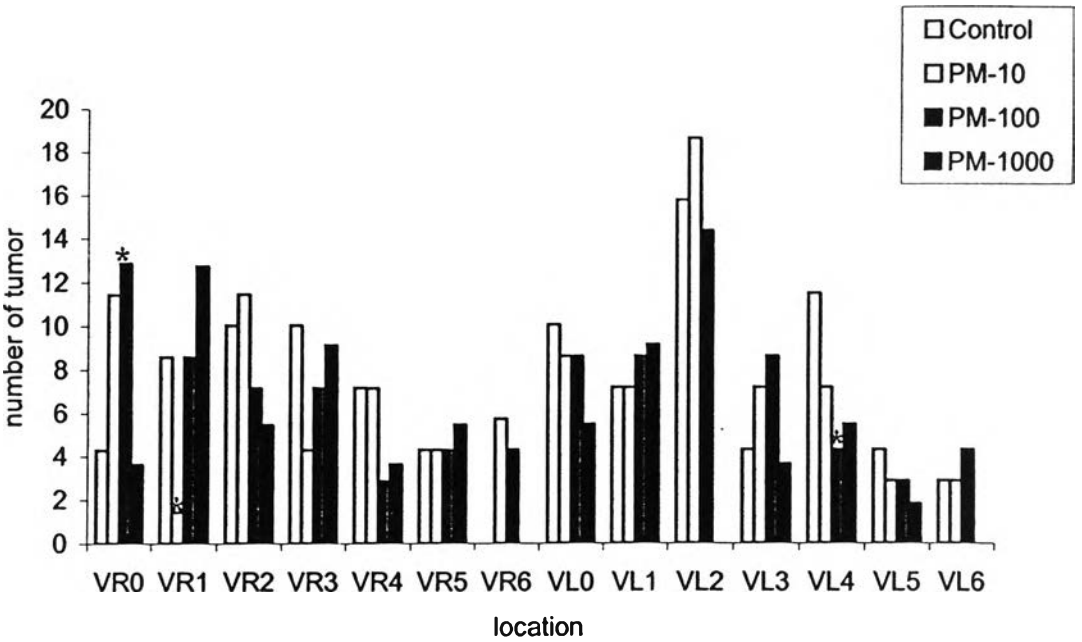


Figure 4.57 Distribution of tumor localization on anti-tumor study of *P. mirifica* on DMBA induced mammary tumor rat at grossly day. The asterisk shows significant difference compared with control at the 0.05 levels.

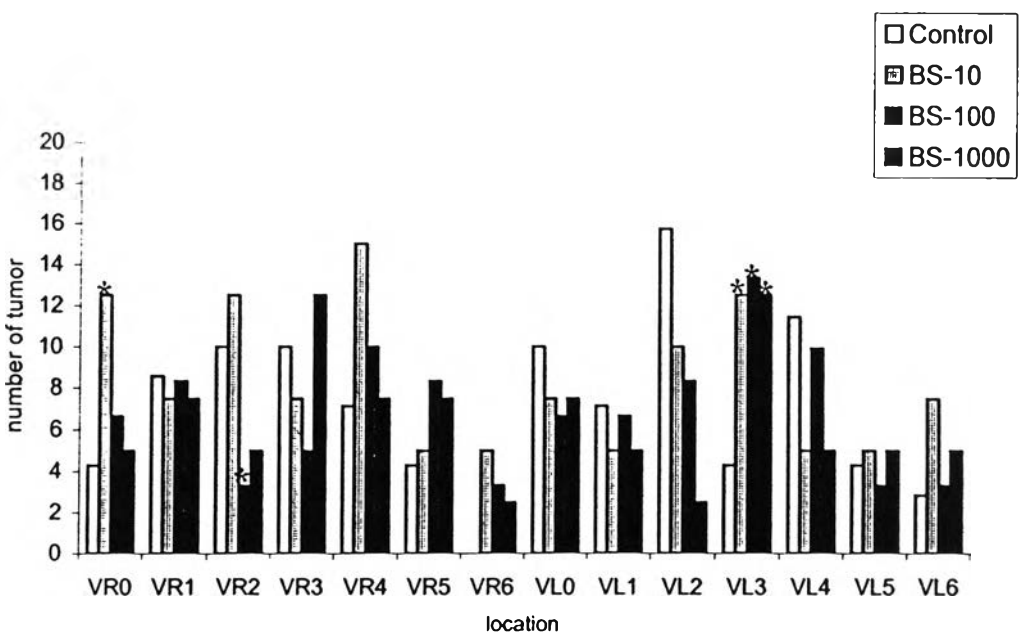


Figure 4.58 Distribution of tumor localization on anti-tumor study of *B. superba* on DMBA induced mammary tumor rat at grossly day. The asterisk shows significant difference compared with control at the 0.05 levels.

2) Tumor growth

Number of mammary tumor

In carcinogen-fed rats, the onset of the first tumor nodule was detectable by palpation as early as the forth weeks after DMBA administration. The responses of tumor-bearing rats to feeding for 28 consecutive days with *P. mirifica* and *B. superba* as compared with the control group are shown in Table 4.12 and Figure 4.59-4.60. Mammary tumors of each group had been found between the forth to sixth weeks after treated with DMBA. The number of tumor were rapidly increased and gone steady at the eleventh weeks.

Comparison of the mean of tumor number since the first to the last week, there were significant difference at least 2 treatments at the seventh to twentieth weeks of experiment by Chi-square at $p<0.05$.

In PM-1000, the tumor number was found lower than other groups. At the seventh to twentieth weeks, the multiplicities of the mammary tumors were presented statistical significant difference ($p<0.05$). The multiplicities of mammary tumors of BS-10 were significant higher than the control group in the seventh weeks as well as the nineteenth weeks of BS-100 group.

Sequential observation data for incidence of palpable mammary tumors after treated with *P. mirifca* and *B. superba*, the antitumor activity are presented in Table 4.13 and Figure 4.61-4.62.

During treatment, tumor number of PM-10 showed slightly growth (data not shown) while as PM-100 and PM-1000 showed more growth than control group. At the second weeks, PM-10 and PM-100 showed significant higher than the control. BS-100 showed significant higher at the second and third weeks.

All of *B. superba* treated groups showed more growth than the control group. After treatment, the numbers of tumor in the control and *P. mirifica* treated groups were declined. However, only PM-1000 treated group showed significant lower than the control group at the eleventh to fourteenth of weeks.

Table 4.12 Mean ± S.E. value of multiplicity of tumor on anti-tumor study of *P. mirifica* and *B. superba* in DMBA-induced mammary tumor rats

Week	Control	PM-10	PM-100	PM-1000	BS-10	BS-100	BS-1000
0	0	0	0	0	0	0	0
1	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0
4	0.1 ± 0.1	0	0.1 ± 0.1	0	0	0.1 ± 0.1	0
5	0.3 ± 0.2	0.5 ± 0.2	0.2 ± 0.1	0.4 ± 0.1	0.3 ± 0.2	0.5 ± 0.3	0.1 ± 0.1
6	1.4 ± 0.3	1.5 ± 0.3	0.9 ± 0.2	0.7 ± 0.2	0.9 ± 0.2	1.5 ± 0.4	0.6 ± 0.2
7	2.2 ± 0.3	2.7 ± 0.4	2.3 ± 0.3	1.8 ± 0.3	2.6 ± 0.4	2.8 ± 0.7	1.6 ± 0.4
8	3.2 ± 0.4	3.9 ± 0.5	3.3 ± 0.3	2.3 ± 0.3	3.4 ± 0.5	3.6 ± 0.7	3.2 ± 0.6
9	4.2 ± 0.4	4.4 ± 0.5	4.6 ± 0.4	3.2 ± 0.4	4.2 ± 0.5	4.4 ± 0.6	3.8 ± 0.6
10	4.5 ± 0.4	4.7 ± 0.5	5.4 ± 0.3	3.5 ± 0.3	4.9 ± 0.5	4.9 ± 0.5	4.6 ± 0.7
11	4.9 ± 0.5	5.4 ± 0.5	5.4 ± 0.4	3.6 ± 0.4	5.4 ± 0.5	5.5 ± 0.4	4.8 ± 0.7
12	4.9 ± 0.5	5.2 ± 0.5	5.3 ± 0.3	3.9 ± 0.6	5.6 ± 0.5	5.6 ± 0.6	4.8 ± 0.8
13	4.9 ± 0.5	4.9 ± 0.5	5.4 ± 0.4	4.2 ± 0.6	5.3 ± 0.4	5.5 ± 0.6	5.1 ± 0.8
14	5.1 ± 0.5	5.1 ± 0.5	5.2 ± 0.3	3.8 ± 0.6	5.2 ± 0.5	5.4 ± 0.6	5.3 ± 0.8
15	4.8 ± 0.5	5.1 ± 0.5	4.8 ± 0.3	3.7 ± 0.6	5.3 ± 0.6	5.8 ± 0.6	5.0 ± 0.8
16	4.8 ± 0.5	5.0 ± 0.5	4.8 ± 0.3	3.4 ± 0.5	5.3 ± 0.6	5.5 ± 0.6	5.0 ± 0.8
17	4.5 ± 0.5	4.8 ± 0.4	4.8 ± 0.3	3.1 ± 0.5*	6.0 ± 0.5*	5.6 ± 0.5	5.4 ± 0.6
18	4.4 ± 0.4	4.9 ± 0.3	4.7 ± 0.3	3.1 ± 0.5*	5.7 ± 0.6	5.6 ± 0.5	5.6 ± 0.6
19	4.6 ± 0.4	5.4 ± 0.6	4.6 ± 0.4	3.0 ± 0.7*	5.3 ± 0.5	5.9 ± 0.5*	5.3 ± 0.6
20	4.5 ± 0.4	5.3 ± 0.6	4.6 ± 0.4	3.1 ± 0.7*	5.3 ± 0.5	5.5 ± 0.3	5.5 ± 0.5

* = Statistical significant at $p<0.05$ compared with control

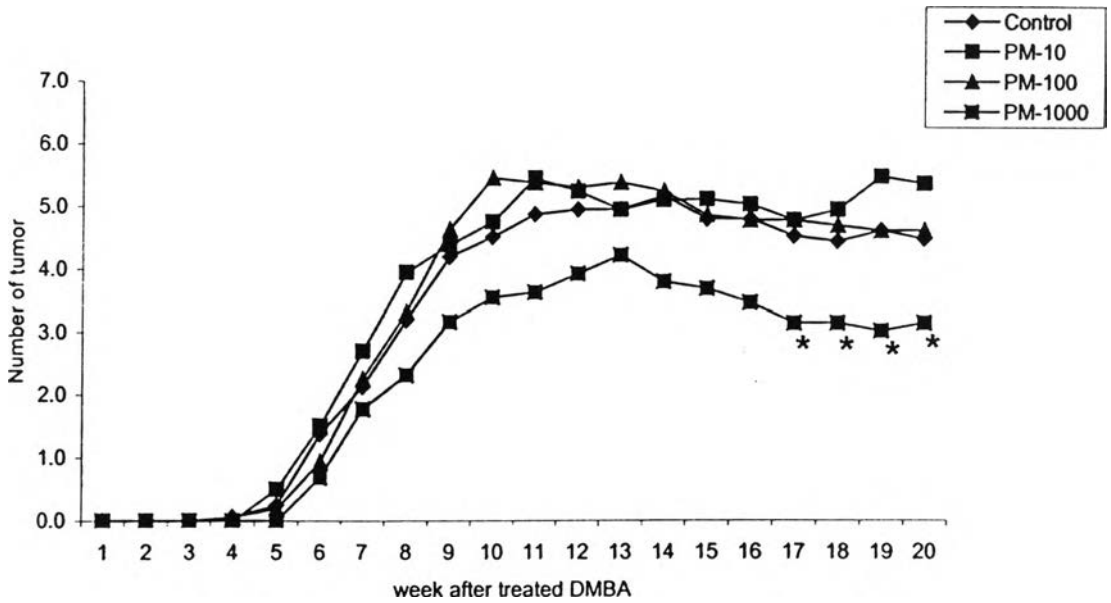


Figure 4.59 Mean ± S.E. value of multiplicity of tumor on anti-tumor study of *P. mirifica* in DMBA-induced mammary tumor rats. The asterisk shows significant difference compared with control at the 0.05 levels.

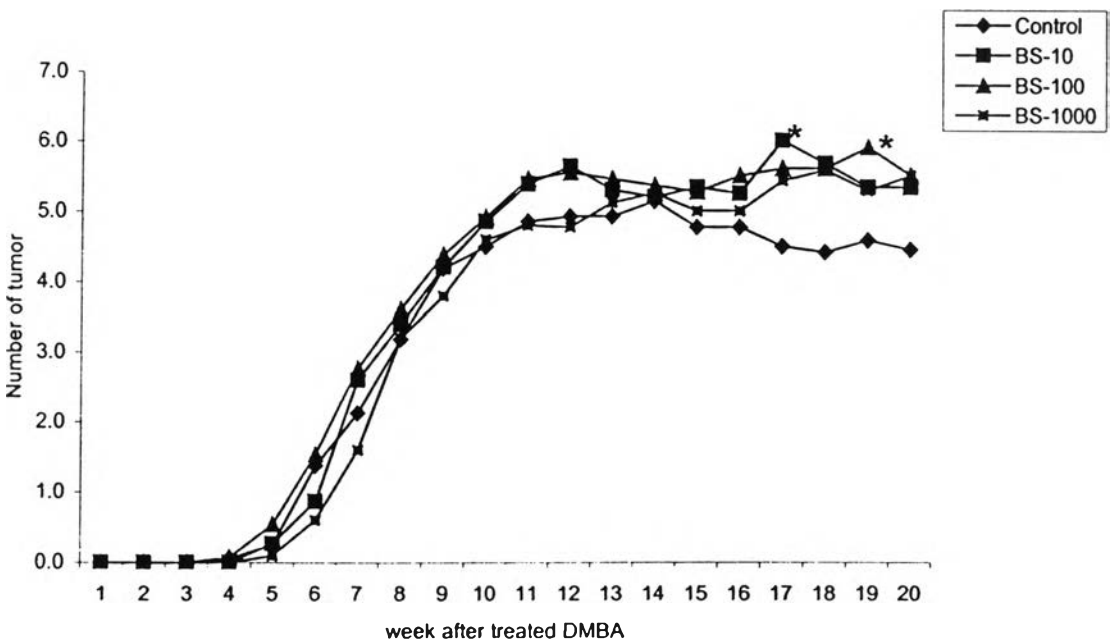


Figure 4.60 Mean ± S.E. value of multiplicity of tumor on anti-tumor study of *B. superba* in DMBA-induced mammary tumor rats. The asterisk shows significant difference compared with control at the 0.05 levels.

Table 4.13 Mean ± S.E. value of multiplicity of tumor on anti-tumor of *P. minfica* and *B. superba* in DMBA-induced mammary tumor rats

Week	Control	PM-10	PM-100	PM-1000	BS-10	BS-100	BS-1000
1	1.3 ± 0.3	1.6 ± 0.2	1.6 ± 0.2	1.5 ± 0.2	1.3 ± 0.1	1.6 ± 0.2	1.1 ± 0.1
2	1.9 ± 0.4	2.8 ± 0.3*	2.9 ± 0.4*	2.1 ± 0.2	2.2 ± 0.3	3.2 ± 0.3*	2.5 ± 0.4
3	3.1 ± 0.4	3.9 ± 0.4	3.9 ± 0.3	2.7± 0.3	4.0 ± 0.4	4.2 ± 0.5*	3.7 ± 0.5
4	4.1 ± 0.4	4.5 ± 0.4	5.0 ± 0.3	3.5 ± 0.5	4.6 ± 0.4	4.8 ± 0.5	4.4 ± 0.6
5	4.3 ± 0.4	4.9 ± 0.5	5.3 ± 0.4	3.6 ± 0.5	5.0 ± 0.4	5.4 ± 0.5	5.1 ± 0.6
6	4.6 ± 0.4	5.3 ± 0.5	5.4 ± 0.4	3.8 ± 0.6	5.5 ± 0.4	5.5 ± 0.5	5.0 ± 0.7
7	4.9 ± 0.5	5.2 ± 0.5	5.4 ± 0.3	4.1 ± 0.6	5.6 ± 0.5	5.5 ± 0.5	5.1 ± 0.8
8	4.9 ± 0.5	5.2 ± 0.5	5.2 ± 0.4	4.2 ± 0.6	5.5 ± 0.5	5.5 ± 0.6	5.1 ± 0.8
9	5.1 ± 0.5	5.2 ± 0.5	5.2 ± 0.4	3.7 ± 0.6	5.2 ± 0.5	5.3 ± 0.6	5.1 ± 0.8
10	4.8 ± 0.5	5.2 ± 0.4	5.0 ± 0.3	3.6 ± 0.5	5.4 ± 0.6	5.3 ± 0.6	5.0 ± 0.8
11	4.8 ± 0.5	5.3 ± 0.4	4.8 ± 0.3	3.4 ± 0.5*	6.0 ± 0.5	5.7 ± 0.6	5.1 ± 0.8
12	4.7 ± 0.5	5.3 ± 0.4	4.8 ± 0.3	3.0 ± 0.6*	5.8 ± 0.7	5.8 ± 0.5	5.8 ± 0.5
13	4.6 ± 0.4	5.4 ± 0.5	4.7 ± 0.3	3.0 ± 0.6*	5.7 ± 0.6	6.0 ± 0.7	6.0 ± 0.5
14	4.6 ± 0.4	5.0 ± 0.4	4.8 ± 0.4	3.4 ± 0.6*	5.3 ± 0.5	6.3 ± 0.8	5.7 ± 0.5
15	4.5 ± 0.4	4.6 ± 0.6	4.8 ± 0.5	4.0 ± 0.7	5.5 ± 0.5	5.8 ± 0.8	5.3 ± 0.6

* = Statistical significant at $p<0.05$ compared with control

NA = Not available rat to record

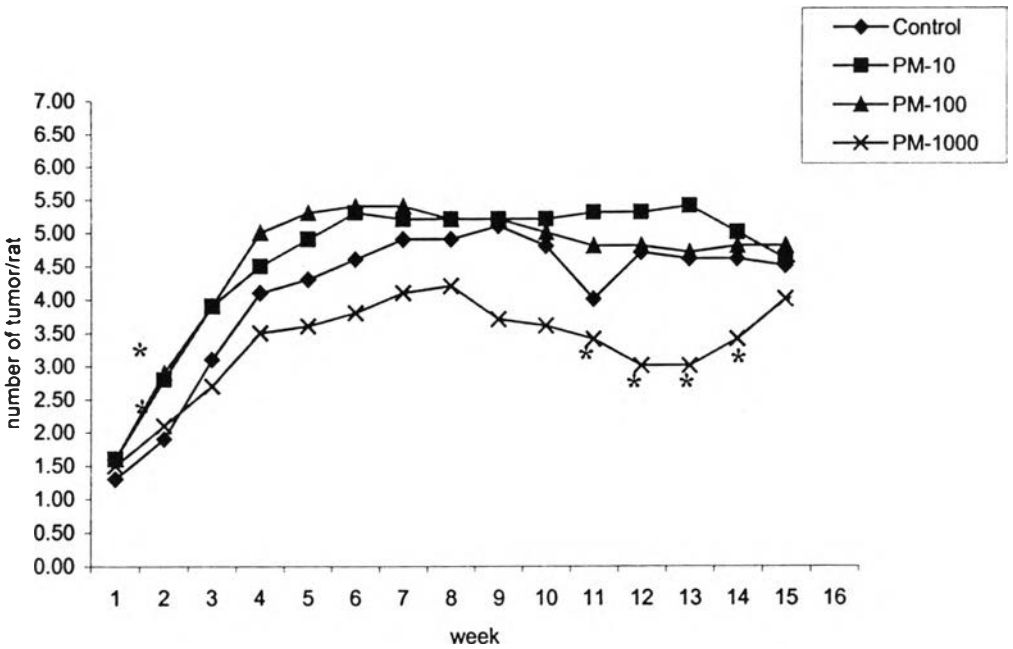
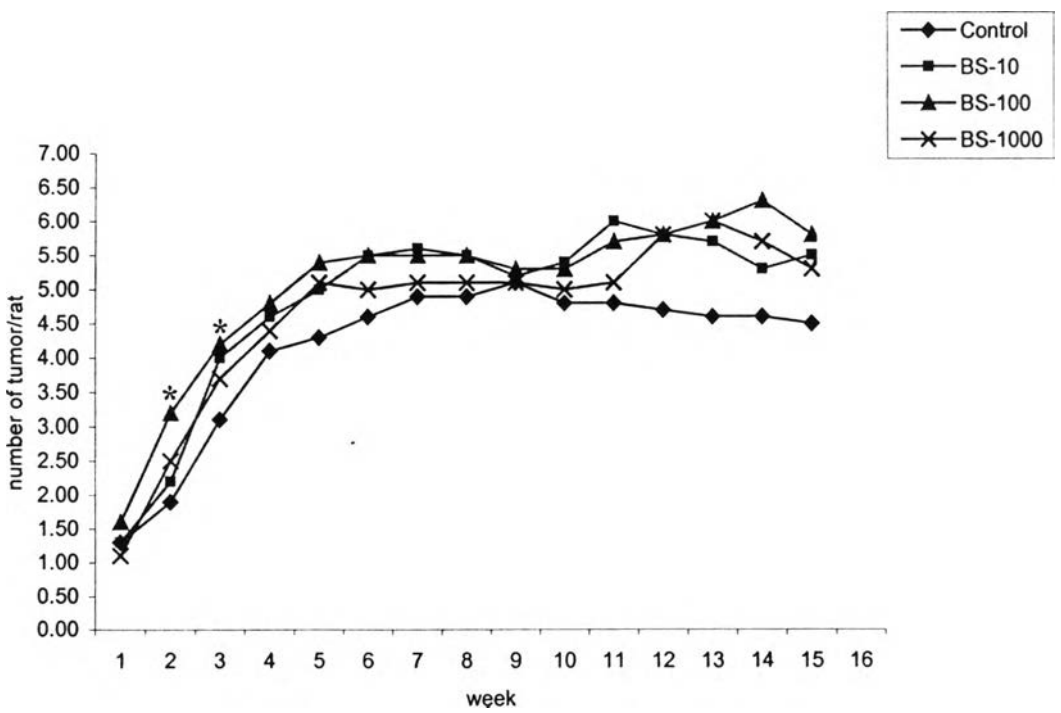


Figure 4.61 Multiplicity of tumor on anti-tumor study of *P. mirifica* in DMBA-induced mammary tumor in rats. The asterisk shows significant difference compared with control at the 0.05 levels.



Mean diameter of mammary tumor

Weekly mean diameter of mammary tumor of tumor-bearing rats treated with 4 consecutive weeks of *P. mirifica* and *B. superba* are shown in Table 4.14 and Figure 4.63-4.64.

The mean diameter of all groups were rapidly increased during the forth to eighth week of experiment and then become stable. There was a statistical significant difference ($p<0.05$) comprising the control group in some weeks as follows. In PM-100, the mean diameter of tumor was found significant lower at the seventh, tenth and fourteenth weeks. In PM-1000, there were significant lower at the seventh to tenth weeks and the fourteenth weeks of experiment.

In *B. superba* treated group, there was a significant lower in BS-1000 group at the seventh and ninth weeks. On the other hand, the mean diameter of tumor showed significant higher in BS-10 at the twelfth to thirteenth and the sixteenth weeks. The mean diameter of tumor in BS-100 was higher than the control group in the thirteenth and fifteenth – sixteenth weeks of experiment.

Considering the effect of *P. mirifica* and *B. superba* on the mean diameter of tumor are presented in Figure 4.65-4.66. During treatment, there was no significant difference comparing with the control group. After treatment, PM-100 and PM-1000 group showed significant lower than the control group in the fifteenth and twelfth weeks, respectively. BS-10 was found significant higher than the control group at the fifth to eleventh and thirteenth of weeks ($p<0.05$).

Table 4.14 Mean diameter of tumor on anti-tumor study of *P. mirifica* and *B. superba* in DMBA-induced mammary tumor rats

Week	Control	PM-10	PM-100	PM-1000	BS-10	BS-100	BS-1000
1	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0
4	0.06±0.06	0	0.06±0.06	0	0	0.06±0.06	0
5	0.23±0.11	0.32±0.10	0.25±0.15	0.00±0.06	0.20±0.09	0.35±0.11	0.15±0.10
6	0.78±0.12	0.74±0.08	0.65±0.11	0.54±0.13	0.86±0.13	0.84±0.12	0.59±0.17
7	1.25±0.08	1.05±0.06	0.95±0.07*	0.83±0.08*	1.31±0.08	1.24±0.10	0.95±0.11*
8	1.33±0.06	1.18±0.05	1.16±0.05	1.06±0.06*	1.49±0.08	1.43±0.08	1.21±0.08
9	1.37±0.07	1.23±0.06	1.33±0.05	1.13±0.07*	1.54±0.06	1.50±0.08	1.17±0.08*
10	1.43±0.07	1.35±0.12	1.22±0.05*	1.15±0.07*	1.62±0.06	1.49±0.07	1.28±0.07
11	1.40±0.07	1.79±0.32	1.31±0.06	1.13±0.07	1.60±0.07	1.45±0.06	1.28±0.08
12	1.34±0.06	1.35±0.07	1.28±0.06	1.22±0.09	1.60±0.08*	1.50±0.07	1.27±0.10
13	1.39±0.06	1.29±0.08	1.25±0.06	1.20±0.09	1.65±0.09*	1.54±0.08*	1.38±0.11
14	1.52±0.16	1.30±0.09	1.20±0.06*	1.18±0.10*	1.58±0.10	1.57±0.07	1.33±0.10
15	1.35±0.09	1.30±0.09	1.19±0.09	1.23±0.12	1.58±0.12	1.65±0.09*	1.37±0.10
16	1.35±0.09	1.37±0.09	1.16±0.09	1.20±0.14	1.75±0.12*	1.68±0.09*	1.48±0.12
17	1.35±0.10	1.29±0.10	1.20±0.09	1.14±0.15	1.63±0.10	1.62±0.09	1.46±0.11
18	1.37±0.10	1.43±0.11	1.22±0.11	1.09±0.14	1.70±0.11	1.64±0.10	1.48±0.10
19	1.36±0.10	1.35±0.11	1.14±0.17	1.18±0.15	1.62±0.10	1.57±0.10	1.55±0.14
20	1.32±0.12	1.34±0.12	1.16±0.11	1.23±0.18	1.56±0.15	1.42±0.10	1.51±0.13

* = Statistical significant at $p<0.05$ compared with control

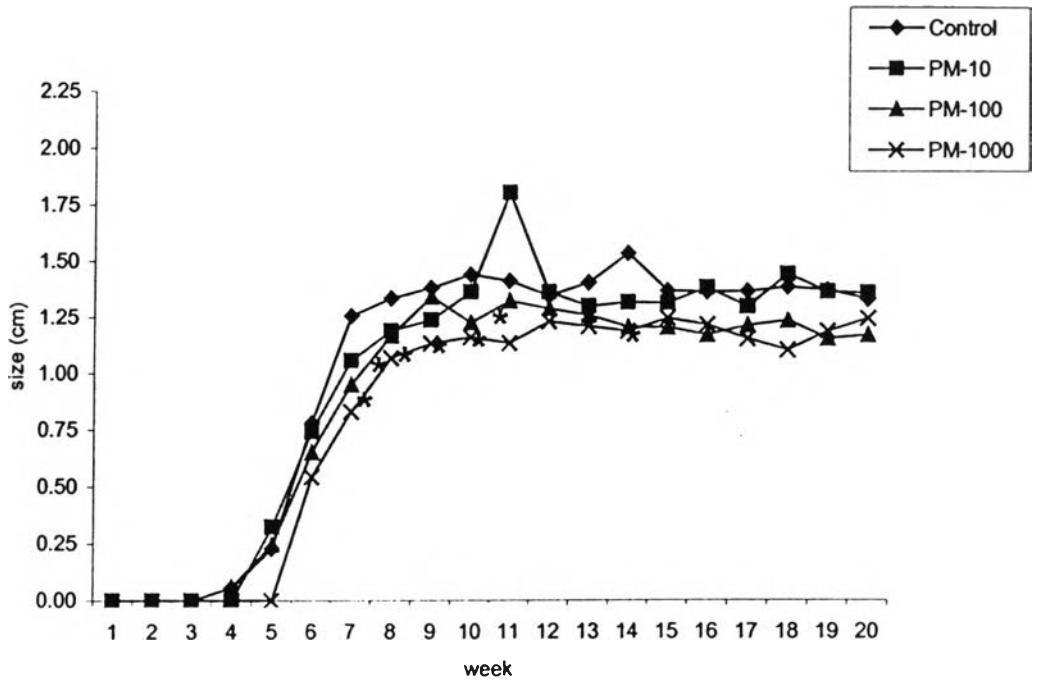


Figure 4.63 Mean diameter of tumor on anti-tumor study of *P. minifica* in DMBA-induced mammary tumor rats. The asterisk shows significant difference compared with control at the 0.05 levels.

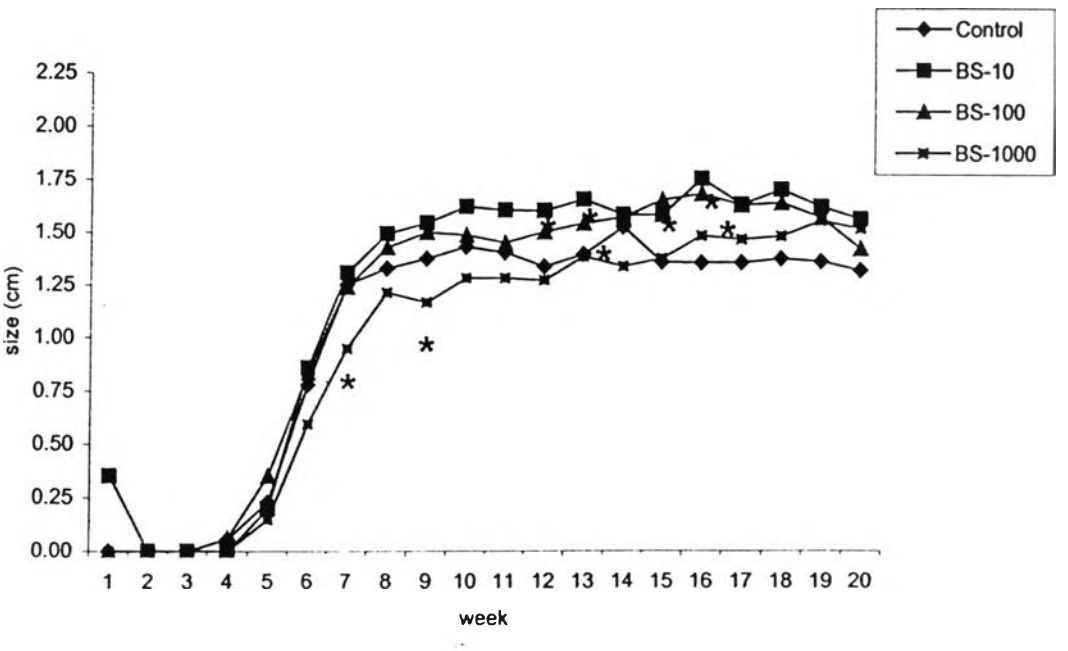


Figure 4.64 Mean diameter of tumor on anti-tumor study of *B. supeba* in DMBA-induced mammary tumor rats. The asterisk shows significant difference compared with control at the 0.05 levels.

Table 4.15 Mean diameter of tumor in DMBA-induced mammary tumor rats after administrated with *P. mirifica* and *B. superba*.

Week	Control	PM-10	PM-100	PM-1000	BS-10	BS-100	BS-1000
1	0.24±0.04	0.22±0.04	0.21±0.04	0.32±0.06	0.24±0.08	0.17±0.04	0.17±0.05
2	0.57±0.07	0.48±0.06	0.48±0.06	0.51±0.06	0.53±0.07	0.58±0.10	0.50±0.07
3	0.85±0.08	0.80±0.07	0.78±0.07	0.73±0.09	1.03±0.12	0.93±0.08	0.79±0.09
4	1.08±0.08	0.90±0.07	1.07±0.06	0.97±0.07	1.21±0.12	1.19±0.09	0.95±0.09
5	1.18±0.08	1.11±0.11	1.13±0.06	1.00±0.09	1.47±0.13*	1.39±0.08	1.16±0.08
6	1.21±0.08	1.17±0.08	1.21±0.06	1.03±0.09	1.54±0.14*	1.34±0.08	1.18±0.09
7	1.27±0.08	1.44±0.23	1.22±0.06	1.18±0.09	1.69±0.16*	1.38±0.08	1.27±0.09
8	1.47±0.17	1.17±0.09	1.20±0.06	1.18±0.09	1.69±0.19*	1.45±0.09	1.25±0.10
9	1.35±0.08	1.18±0.09	1.19±0.07	1.18±0.12	1.73±0.20*	1.43±0.09	1.28±0.10
10	1.40±0.09	1.23±0.09	1.22±0.08	1.15±0.12	1.82±0.24*	1.48±0.10	1.35±0.11
11	1.31±0.09	1.31±0.10	1.22±0.10	1.21±0.15	1.92±0.27*	1.56±0.10	1.39±0.12
12	1.39±0.10	1.37±0.10	1.20±0.09	1.05±0.15*	1.59±0.09	1.53±0.10	1.47±0.13
13	1.36±0.09	1.31±0.10	1.20±0.09	1.09±0.15	1.67±0.12*	1.54±0.11	1.51±0.11
14	1.44±0.11	1.33±0.13	1.17±0.11	1.23±0.18	1.62±0.15	1.54±0.12	1.49±0.14
15	1.55±0.17	1.26±0.21	1.09±0.12*	1.36±0.20	1.70±0.23	1.37±0.12	1.59±0.20

* = Statistical significant at $p<0.05$ compared with control

The plants were treated on the 1^{st} – 4^{th} week.

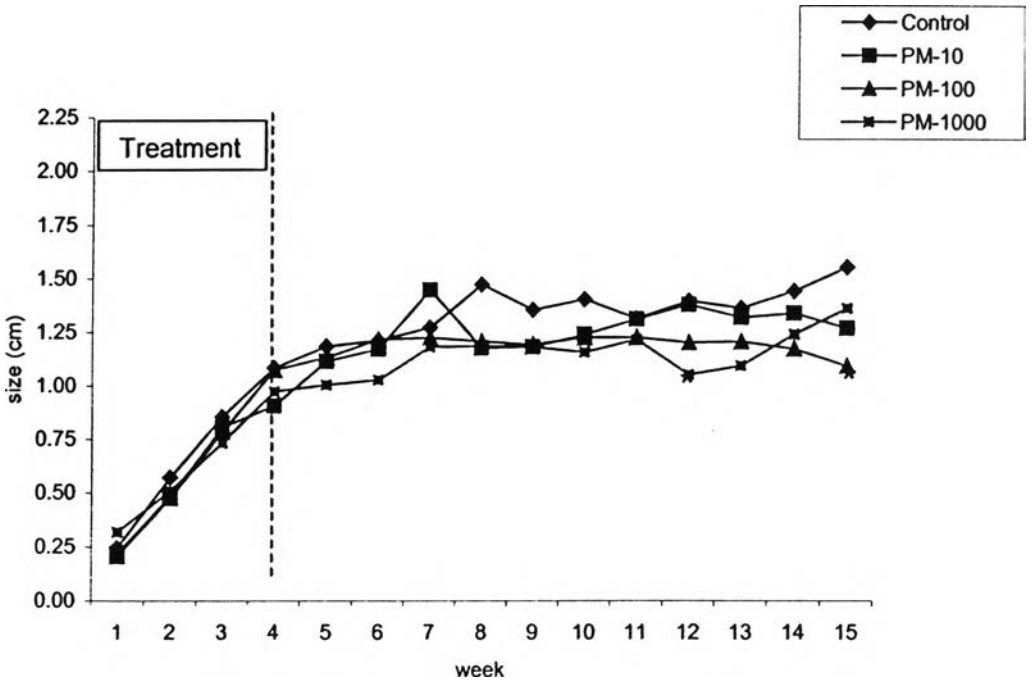


Figure 4.65 Mean diameter of tumor in DMBA-induced mammary tumor rats after administrated with *P. mirifica.* The asterisk shows significant difference compared with control at the 0.05 levels.

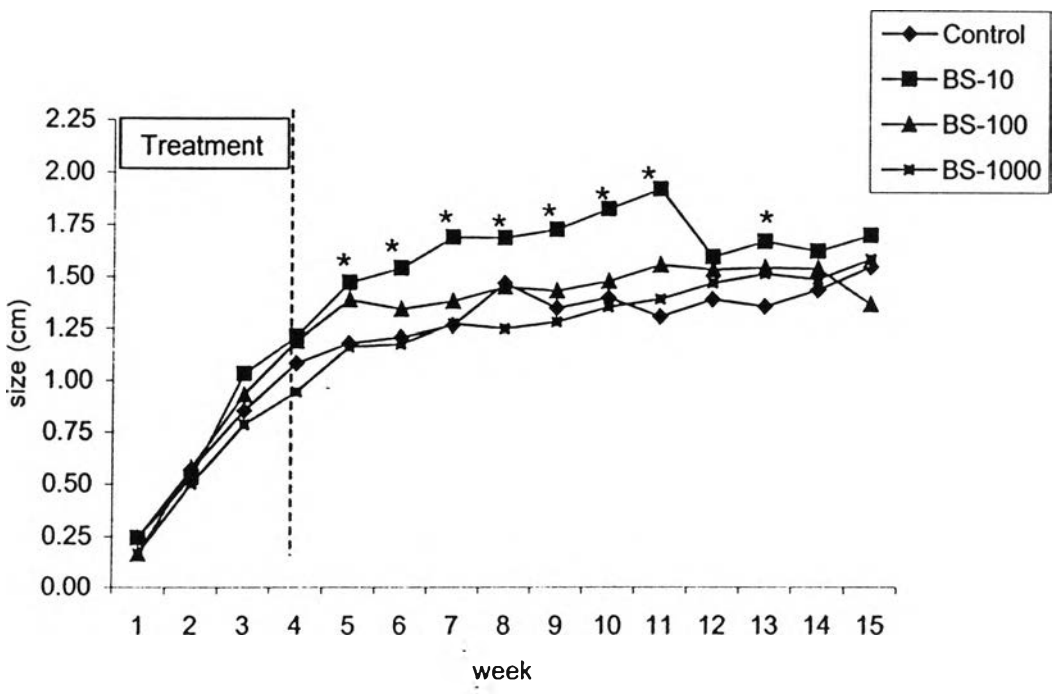


Figure 4.66 Mean diameter of tumor in DMBA-induced mammary tumor rats after administrated with *B. superba.* The asterisk shows significant difference compared with control at the 0.05 levels.

3) Tumor size at grossly day

At the end of experiment, all rats were sacrificed and tumors were evaluated for the multiplicity, weight and size. Total multiplicity or number of tumor per rat was significantly difference lower only in PM-1000 group comparing with the control group. Mean±S.E. of multiplicity showed the significant lower in PM-1000 ($p<0.05$) in the left site while the BS-100 was found higher in the right site. In BS-10 group, the total of tumor per rat was also found significant higher than the control group. Tumor diameter was statistical significant higher than the control group in BS-10. The others were not found significant differences. (Table 4.16 and Figure 4.67-4.70)

Table 4.16 Anti-tumor study of *P. mirifica* and *B. superba* treatment on DMBA-induced mammary tumor in SD rats

Group	Multiplicity			Tumor weight (g)	Tumor Diameter	Tumor Volume (cm^3)
	Left	Right	Total			
Control	3.00±0.29	2.00±0.33	5.00±0.47	2.97±0.64	1.24±0.10	2.96±0.75
PM-10	2.17±0.54	1.50±0.43	3.67±0.67	3.41±1.17	1.39±0.13	4.34±1.22
PM-100	2.50±0.29	2.32±0.26	4.83±0.49	3.39±0.79	1.23±0.23	3.85±1.15
PM-1000	1.13±0.23*	2.25±0.37	3.38±0.42*	4.27±0.73	1.51±0.12	3.91±0.99
BS-10	3.20±0.20	3.60±0.24*	6.80±0.20*	4.28±0.89	1.55±0.78*	5.27±1.06
BS-100	2.75±0.25	2.38±0.26	5.13±0.30	2.73±0.42	1.47±0.10	4.19±0.98
BS-1000	2.67±0.49	2.83±0.40	5.50±0.76	3.52±0.64	1.46±0.11	4.49±0.87

* = Statistical significant at $p<0.05$ compared with control

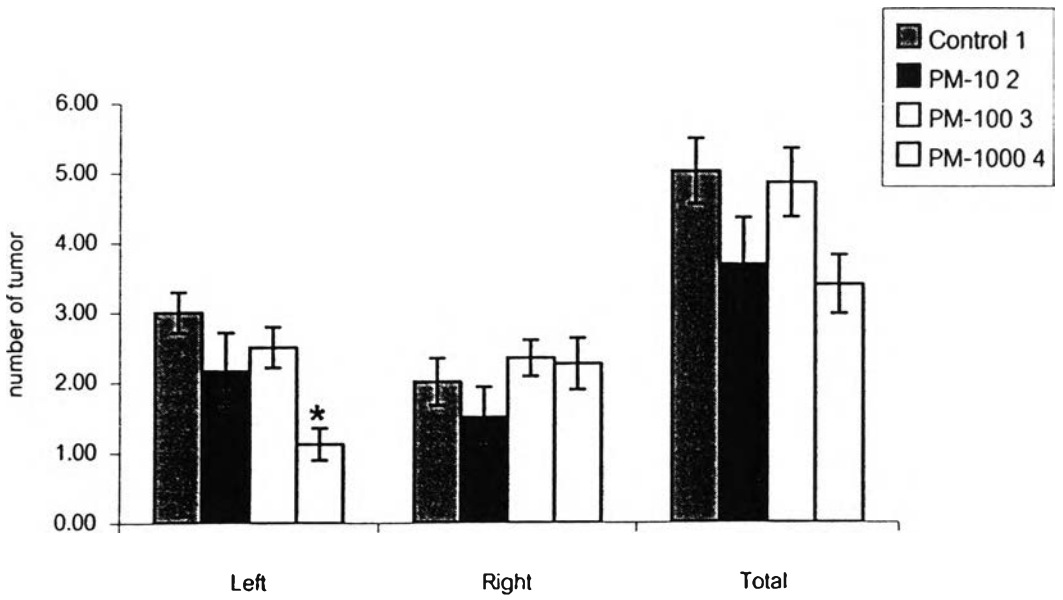


Figure 4.67 Multiplicity of tumor on anti-tumor study of *P. mirifica* in DMBA-induced mammary tumor rats.

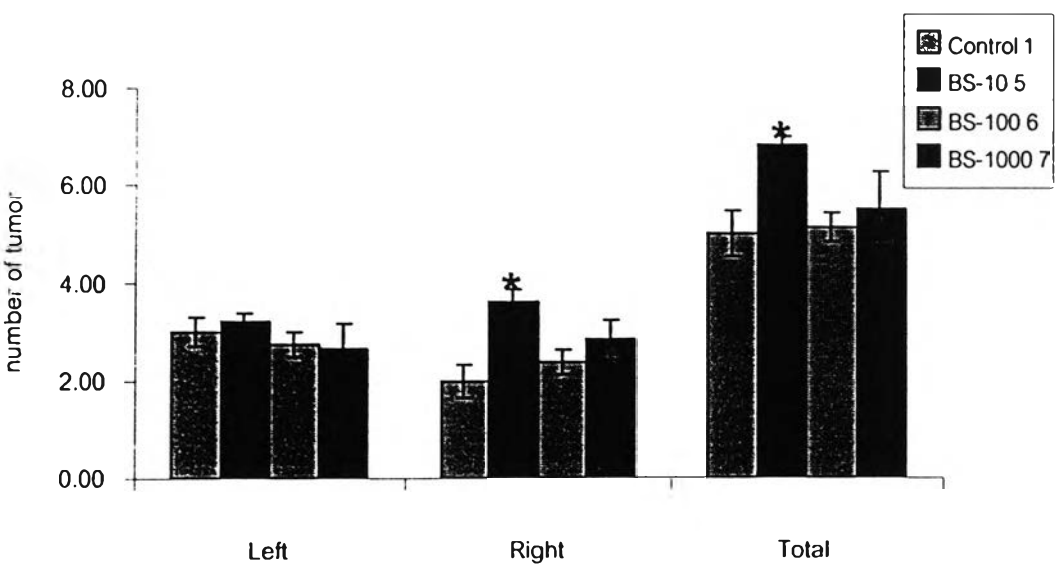


Figure 4.68 Multiplicity of tumor on anti-tumor study of *B. superba* in DMBA-induced mammary tumor rats

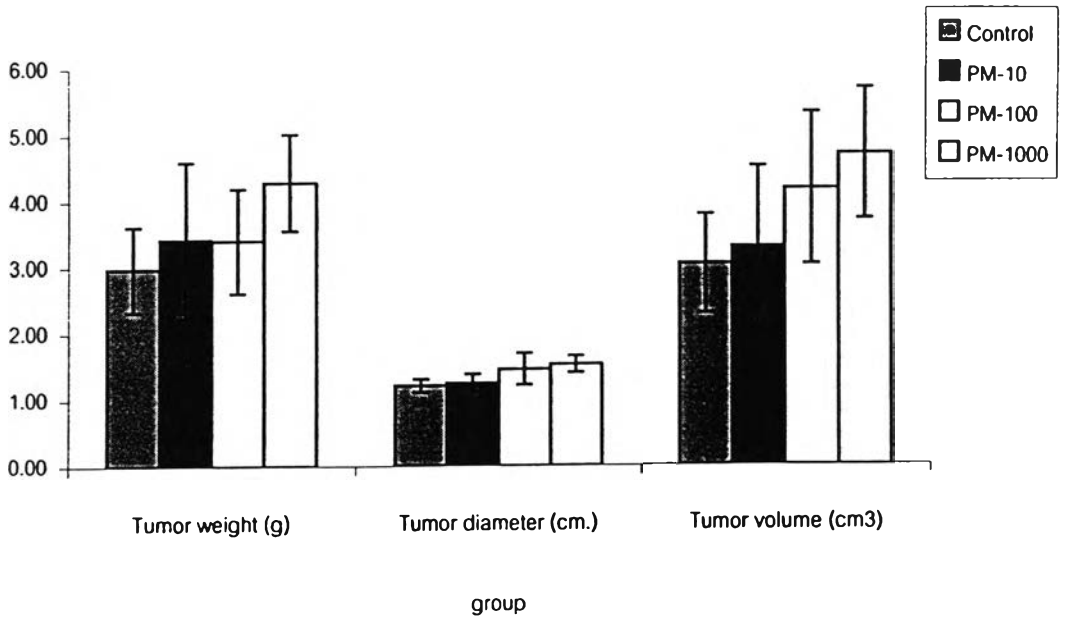


Figure 4.69 Weight, diameter and volume of tumor on anti-tumor study of *P. mirifica* in DMBA-induced mammary tumor rats

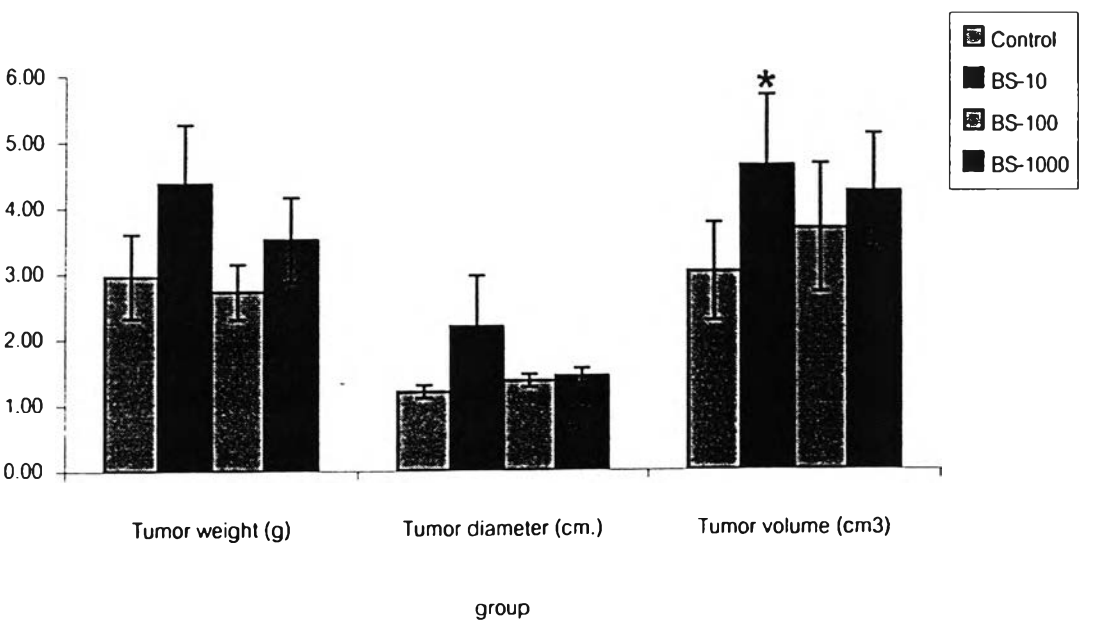


Figure 4.70 Weight, diameter and volume of tumor on anti-tumor study of *B. superba* in DMBA-induced mammary tumor rats

4) Histological study of mammary tumor

Histopathological examination of tumors was classified referring to WHO (Young and Hallowes, 1973). All tumors was malignant which characterized into 5 types; adenocarcinoma, papillary carcinoma, anaplastic carcinoma, cribiform carcinoma and comedo carcinoma (Figure 4.71). Most of them were adenocarcinoma (Figure 4.71A), which showed in varied patterns. Acini were varied in size and shape and lined by 1-2 layer of cuboidal epithelial, which was called as simple adenocarcinoma. More complexity of adenocarcinoma was also found. Papillary carcinoma was accompanied by an epithelial proliferation. The growth of loose and oedesmatous fibrovascular stroma and irregular spaces composing with the branching papillary fronds have occurred (Figure 4.71B). Anaplastic carcinoma was consisted with irregular sheets of epithelial cell arrangement and little stroma (Figure 4.71C). Cribiform carcinoma demonstrated the proliferation of epithelial cell in circumscribed and some secretion into acini can be occurred (Figure 4.71D). Some tumor was found alveolar adenocarcinoma with comedo pattern that central tissue necrosis is surrounded by glandular structures (Figure 4.71E). The spindle cell carcinoma could be found accompanying with adenocarcinoma. (Figure 4.71F). Tumor was found as adenocarcinoma in all groups. The minority of tumor was papillary carcinoma. It is noticeable that the tumor was demonstrated in mixing of tumor pattern. In BS-treated group, it was found as the comedo pattern.

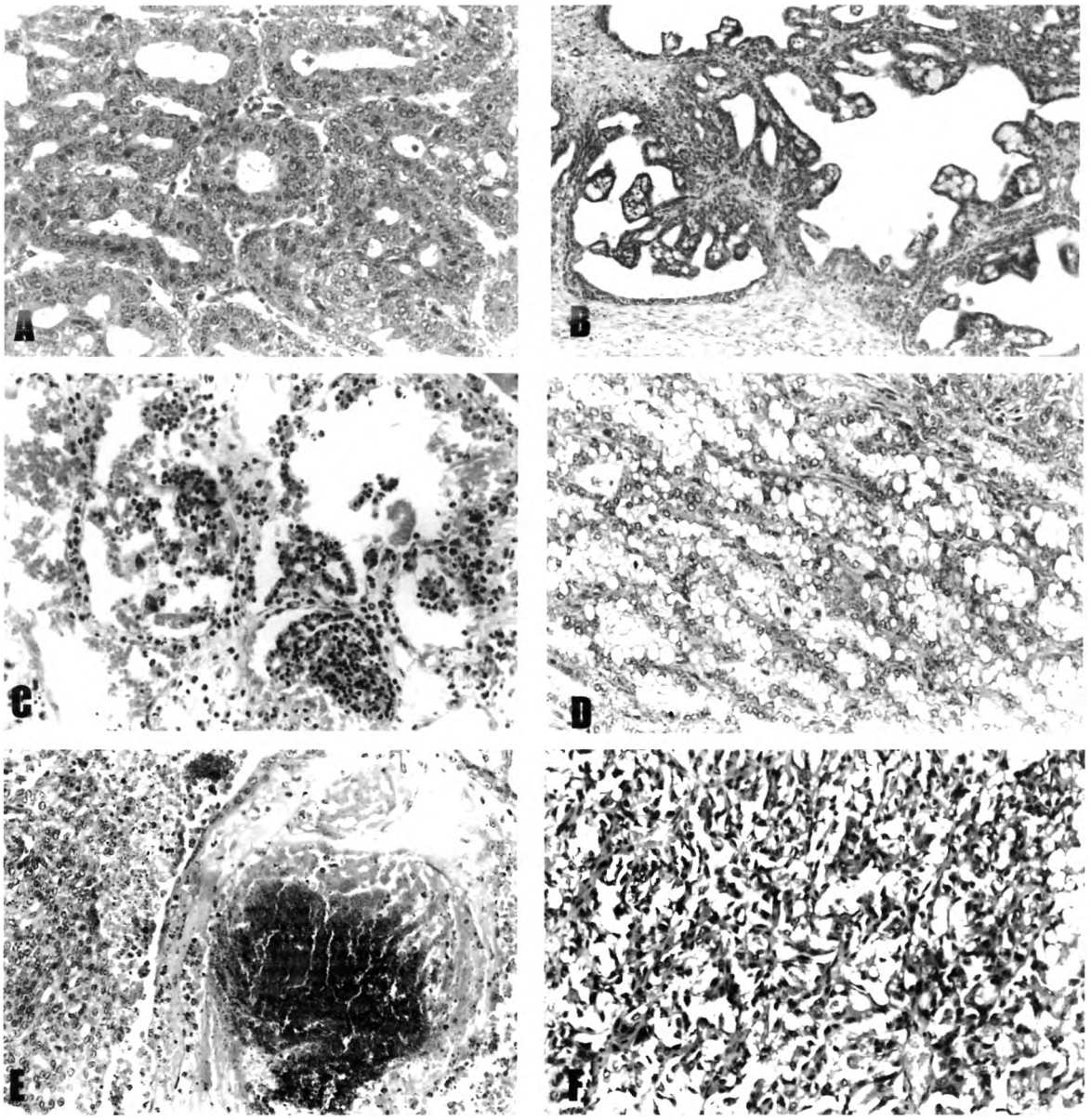


Figure 4.71 Histology of DMBA-induced mammary tumor in rat on anti-tumor study of *P. mirifica* and *B. superba* followed the classification of WHO (Young and and Hallowes, 1973). Magnification x40.

A = Adenocarcinoma
B = Papillary carcinoma
C = Anaplastic carcinoma
D = Cribriform carcinoma
E = Comedocarcinoma
F = Spindle cell carcinoma

4.2.4 Estrogen receptor immunohistochemical in mammary tumor

The evaluation of results was determined by a semiquantitative method. In both of ER-α and β was confined to the nuclei of all cell types. For the negative controls, no specific staining was observed as shown in Figure 4.37. Both receptors were found in mammary tumor compartment. However, there was difference in the staining intensity and localization. Epithelial compartment was found a weak staining intensity with all groups.

All the semi quantitative results are shown by their level of staining intensity as shown in Table 4.17 and Figure 4.72. In most groups, estrogen receptor was found as moderate staining intensity in both subtypes. It is notice that both estrogen subtype was a weak-staining intensity in PM-1000, BS-10 and BS-1000 group. The lowest ratio of both estrogen receptors was found in PM-1000 group.

Table 4.17 Staining intensity in different compartment of mammary tumor, for estrogen receptor subtypes; alpha and beta immunostaining

Group	Treatment	No. of rats	ER-α	ER-β	Ratio
1	Control	5	37.18	44.98	0.83
2	PM-10	5	33.37	42.61	0.78
3	PM-100	5	22.59	32.76	0.69
4	PM-1000	5	12.51	34.35	0.36
5	BS-10	5	17.33	31.87	0.54
6	BS-100	5	21.54	30.53	0.71
7	BS-1000	5	5.55	9.75	0.57

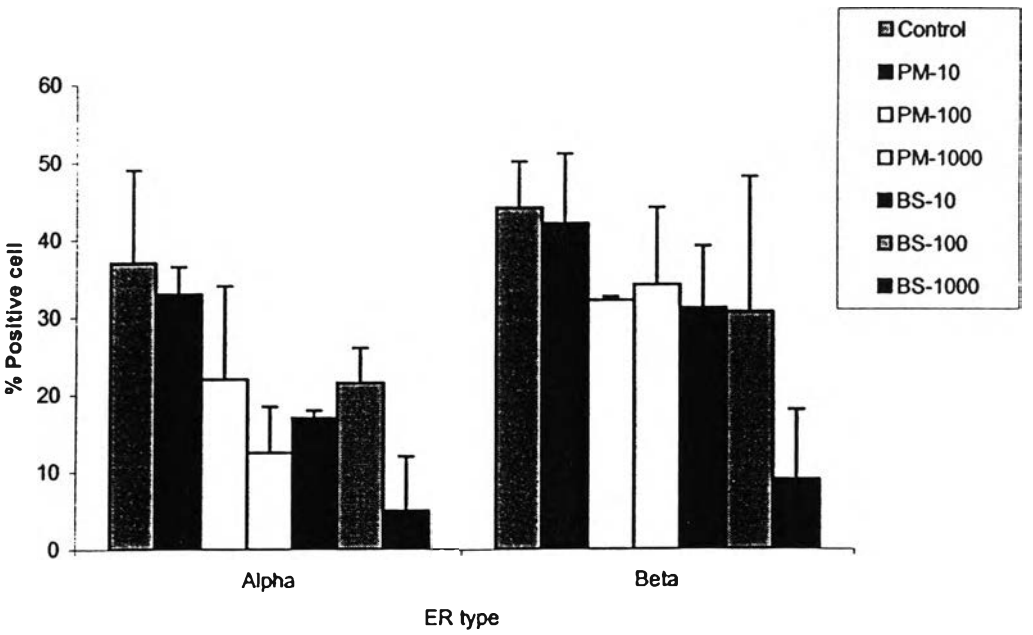


Figure 4.72 Percentage of positive cell staining of mammary tumor for estrogen receptor subtypes; alpha and beta immunostaining

4.3 Quantitative of phytoestrogens in *P. mirifica*, *B. superba*, rat food

4.3.1 Establishing standard curve

The selected method, HPLC-UV monitoring mode was successfully applied for the quantitative determination of the five isoflavones; puerarin, daidzin, genistin, daidzein and genistein in plant, rat food and serum. The chromatogram was shown in Figure 4.73. The quantitative analysis of 5 isoflavones was measured by standard curve of each chemical (Figure 4.74-4.78).

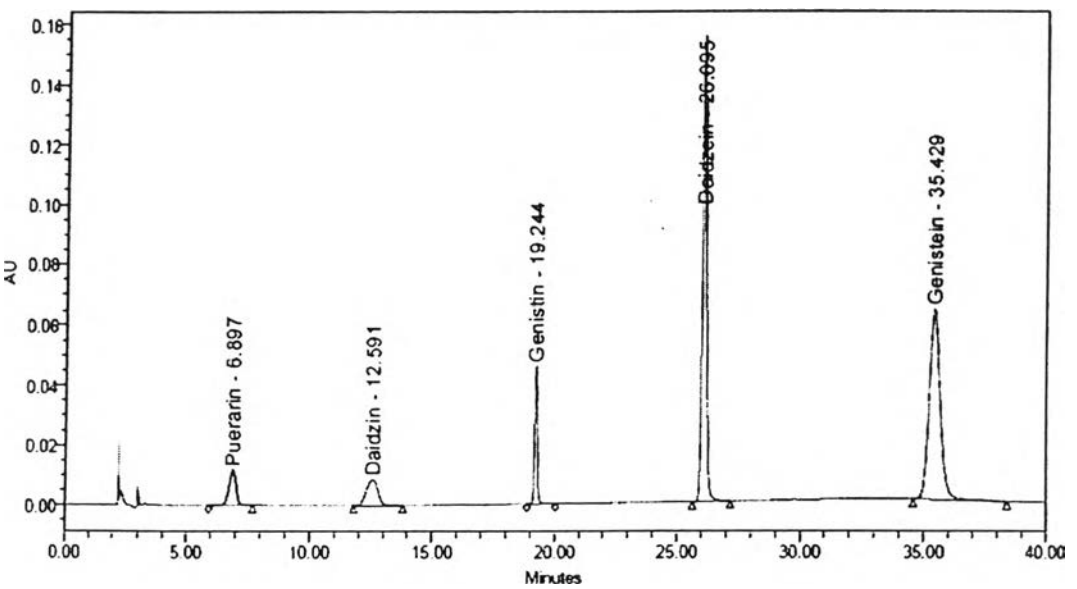


Figure 4.73 The chromatograms of standard puerarin, daidzin, genistin, daidzein and genistein. Symmetry C_{18} reversed column was used to separate the aglycones and their glucuronide products. The elution and gradient conditions were described in the method.

R- Square of puerarin, daidzin, genistin, daidzein and genistein were 0.964, 0.974, 0.934, 0.984 and 0.895, respectively. The linear equation of each standard was shown in Figure 4.39-4.43

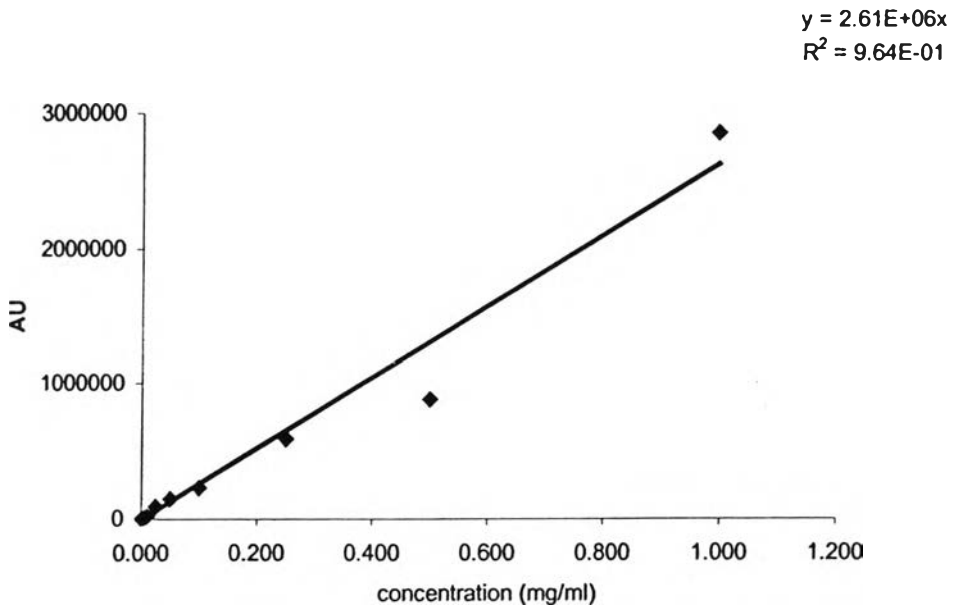


Figure 4.74 Standard curve of Puerarin, AU at 254 nm

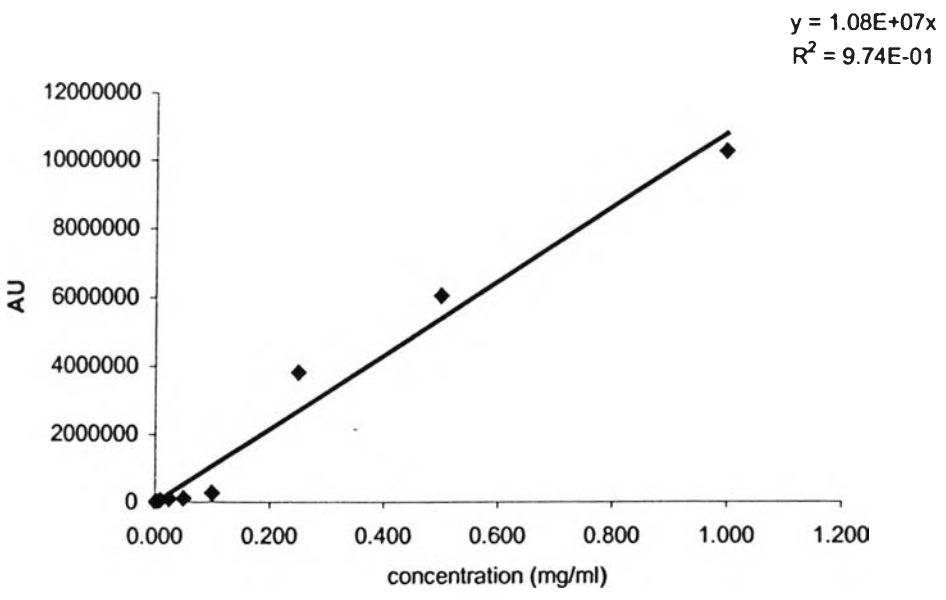


Figure 4.75 Standard curve of Daidzin, AU at 254 nm

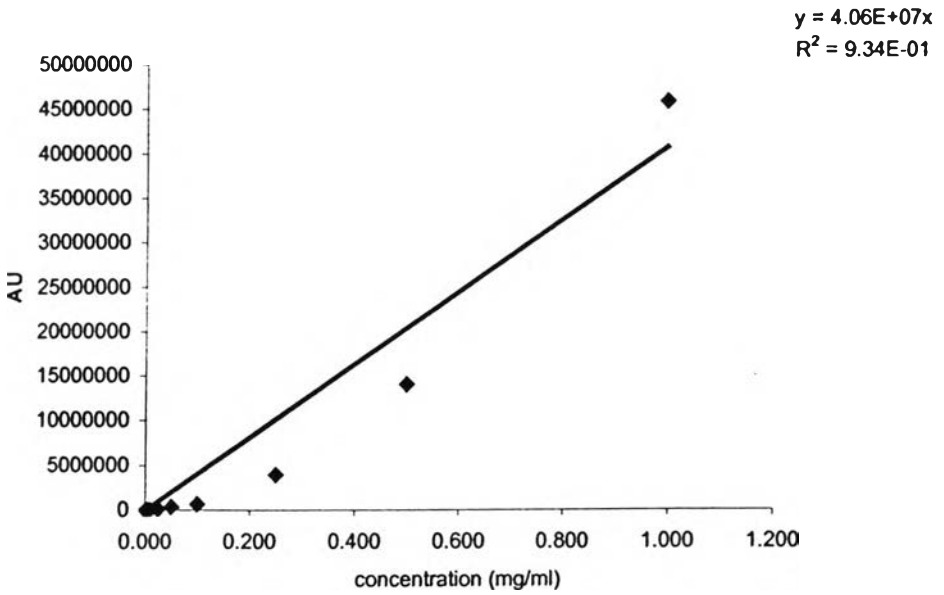


Figure 4.76 Standard curve of Genistin, AU at 254 nm

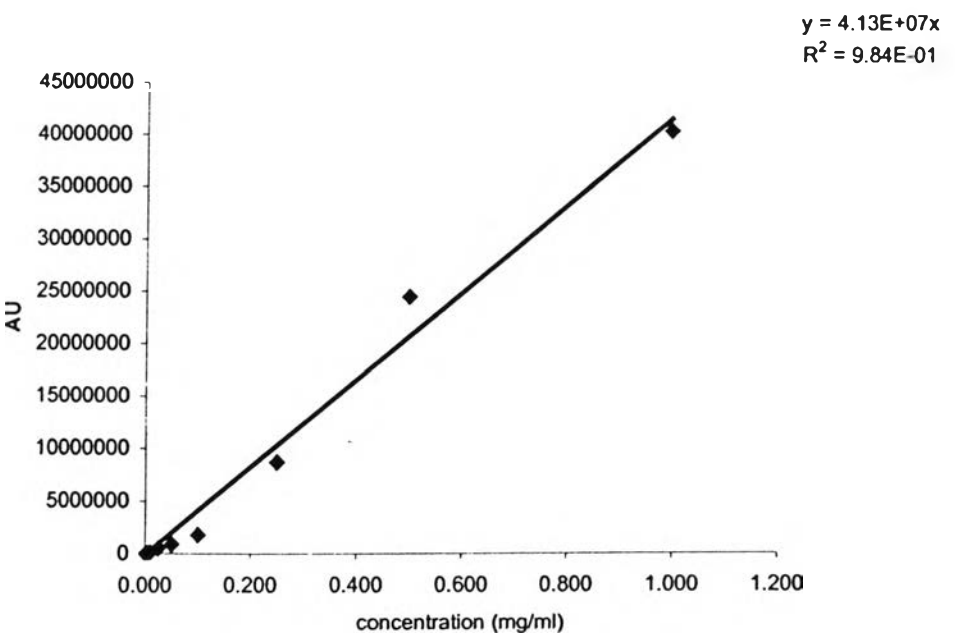


Figure 4.77 Standard curve of Daidzein, AU at 254 nm

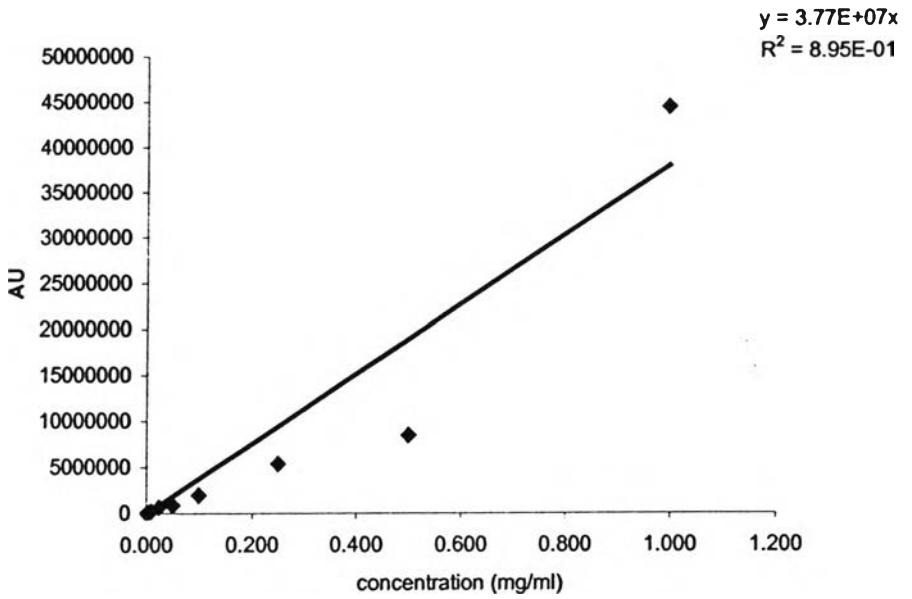


Figure 4.78 Standard curve of Genistein, AU at 254 nm

4.3.2 HPLC analysis of isoflavone in *P. mirifica*, *B. superba* and rat food

The methanolic extracts of *P. mirifica* and *B. superba* powder and rat food were determined with HPLC-UV analysis. The area under the curve of each sample was compare with the standard curve at 254 nm. (Table 4.18)

P. mirifica contained isoflavone in the largest amount of 96.03±0.49 which was classified into by daidzin, genistin, daidzein and genistein, respectively. *B. superba* was found only genistin in the amount of 4.57±0.32 mg/100 g. In rat food, genistin was found in the amount of 152.50±1.01 mg/100 g powder.

Table 4.18 The result of quantification analysis of isoflavone in *P. mirifica* and *B. superba* power and rat food extract determined by HPLC analysis (mg/100 g powder)

Sample/isoflavone	Puerarin	Daidzin	Genistin	Daidzein	Genistein
P. mirifica	96.03±0.49	25.21±2.57	21.46±1.23	9.17±0.22	2.78±0.30
B. superba	N.D.	N.D.	4.57±0.32	N.D.	N.D.
Rat food	N.D.	N.D.	152.50±1.01	6.88±0.14	8.80±0.56

N.D. = Not detected, The data represent the mean and standard deviation (n = 5).

* = Statistical significant at $P < 0.05$ level.

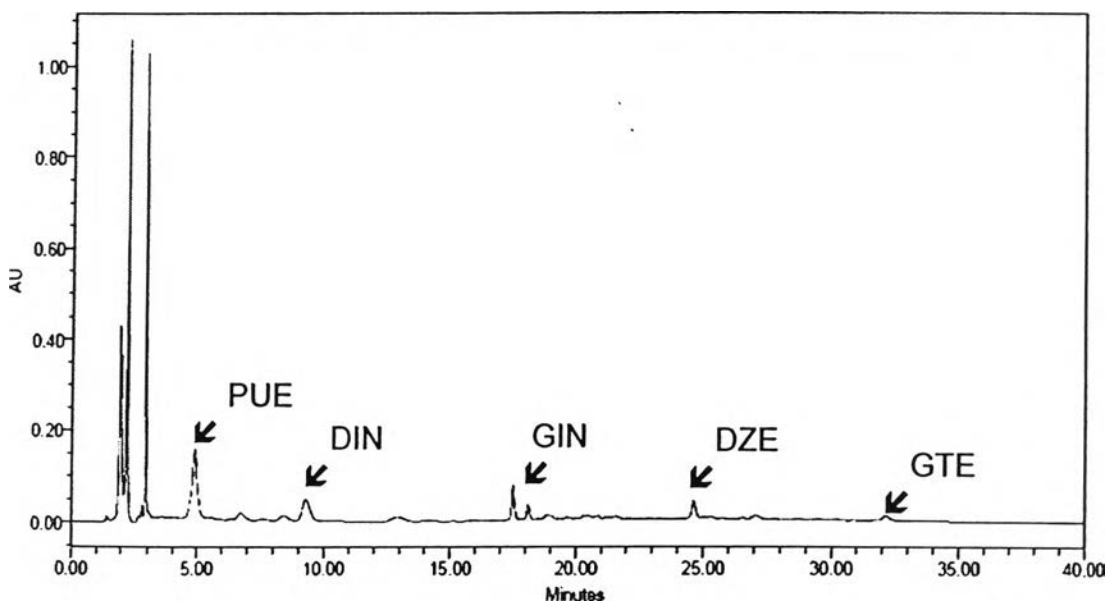


Figure 4.79 The chromatograms from HPLC analysis of methanol of *P. mirifica* was compared with standard puerarin (PUE), daidzin (DIN), genistin (GIN), daidzein (DZE) and genistein (GTE).

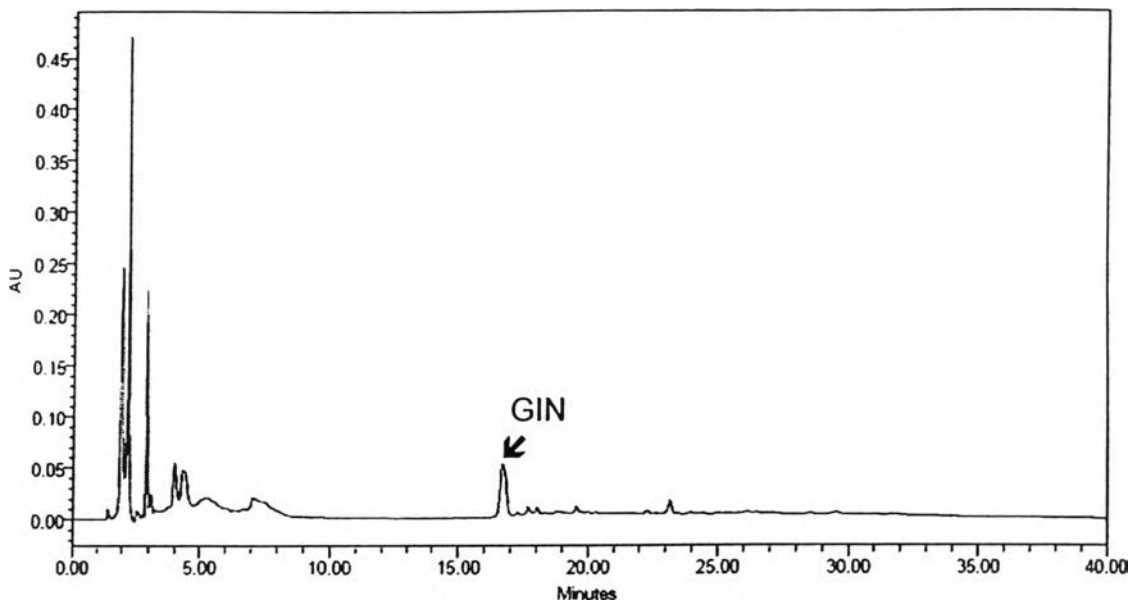


Figure 4.80 The chromatograms from HPLC analysis of methanol of *B. superba* powder were compared with standard puerarin (PUE), daidzin (DIN), genistin (GIN), daidzein (DZE) and genistein (GTE).

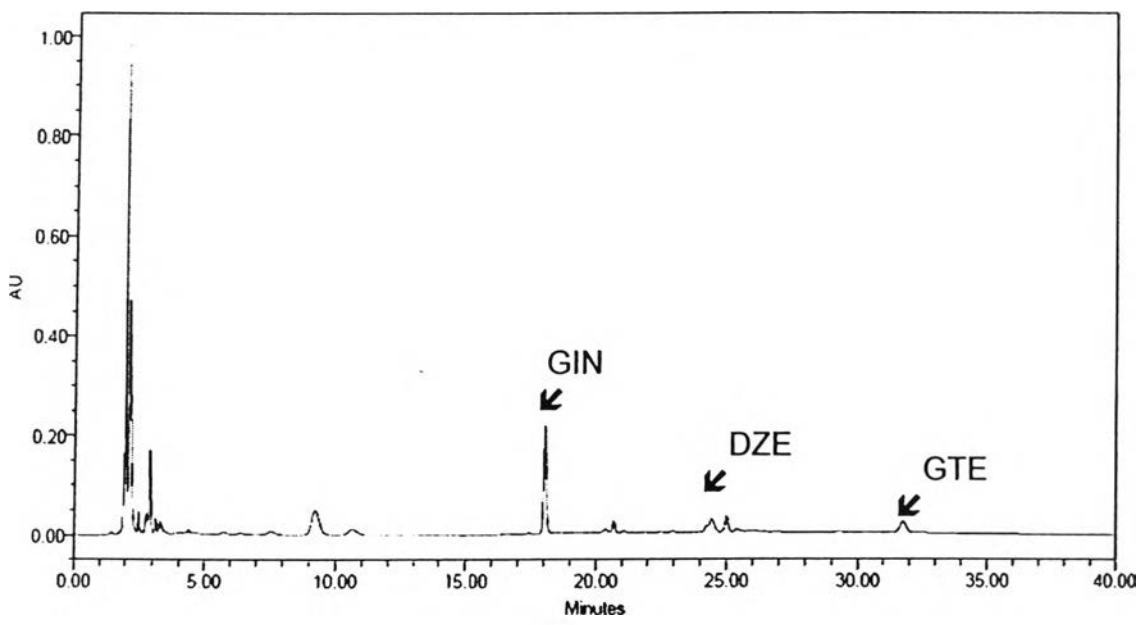


Figure 4.81 The chromatograms from HPLC analysis of methanol of rat food were compared with standard puerarin (PUE), daidzin (DIN), genistin (GIN), daidzein (DZE) and genistein (GTE).