CHAPTER IV

RESULTS

Section 1 Pharmacological activity study

1. Effects of *B. superba* on MAP

There was no significant change in the MAP upon the treatment with various concentration of *B. superba* alcoholic extracts and cavernous nerve stimulation. The MAP of the control group was 105.4 ± 2.5 mmHg (mean ± SE.). The MAP of the animals treated with the extracts at the concentrations of 0.1, 1, 10 and 1,000 mg/kg BW were 107.2 ± 2.2 (n = 10), 112.6 ± 2.8 (n = 10), 109.8 ± 2.9 (n = 10) and 114.2 ± 3.5 (n = 10) mmHg, respectively.

2. Effects of *B. superba* on ICP

This study aimed to investigate for an optimal pre-treatment period of the extract. The animals were pre-treated the extract at the dose of 1,000 mg/kg BW as a single dose for 0.5, 1, 1.5, 2, 2.5 and 3 hr prior to conducting electrical stimulation of the cavernous nerve. The results show that pre-treatment for one hour provided the best response to the electrical stimulation. Therefore, one hour pretreatment of the animals with the extract was employed for the subsequent studies.

Figure 10 demonstrates examples of the ICP recorded from a control animal (Figure 10a) and a 1 mg/kg extract-treated rat (Figure 10b). The electrical stimulation of cavernous nerve of the control rat (DW-treated rat) induced penile erection with an ICP of about 30 mmHg. The extract treatment enhanced the increased ICP to the peak of about 120 mmHg.

2.1 Screening for the most active part and source of *B. superba*.

B. superba collected from two sources (Phayao and Phrae) were studied. DB, FB, DR and FR were extracted with ethanol. This study aimed to examine for the most effective part of *B. superba* in increasing the ICP.

The control animals received only DW exhibited the ICP of about 45 mmHg after being induced by cavernous nerve stimulation (Figure 11). Treatment of the rats with alcoholic extracts of various parts at the doses of 1, 10 or 1,000 mg/kg BW significantly induced increases in the ICP compared with the control group. The patterns of the responses did not follow concentration-response relationship, i.e. higher extract concentrations did not produce greater responses. However, in most cases, the extract at the concentration of 1 mg/kg induced the highest increases in the ICP. The extracts of DRPr revealed the highest activity (Figure 11). Therefore, this extract was used in all the subsequent studies.

2.2 Dose-response relationship of *B. superba* extracts

The extracts of DRPr were employed to study for a dose-response relationship. It is interesting that the response curve appeared as a bell shape (Figure 12). That is the response followed the rule of dose-response relationship at the low extract concentrations (0.1-1 mg/kg) with the maximum effect at the dose of 1 mg/kg. After then the increased ICP were reduced when the extract concentrations were increased (Figure 12).

2.3 Dose-response relationship of *B. superba* extracts on chronic treatment

After exposing to the various dosages of extract daily for 6 months, the ICP was recorded. The results showed that the increases in the ICP were similar to

those of the animals receiving the extract only once (Figure 13). In addition, the maximum response was also observed at the dose of 1 mg/kg BW.

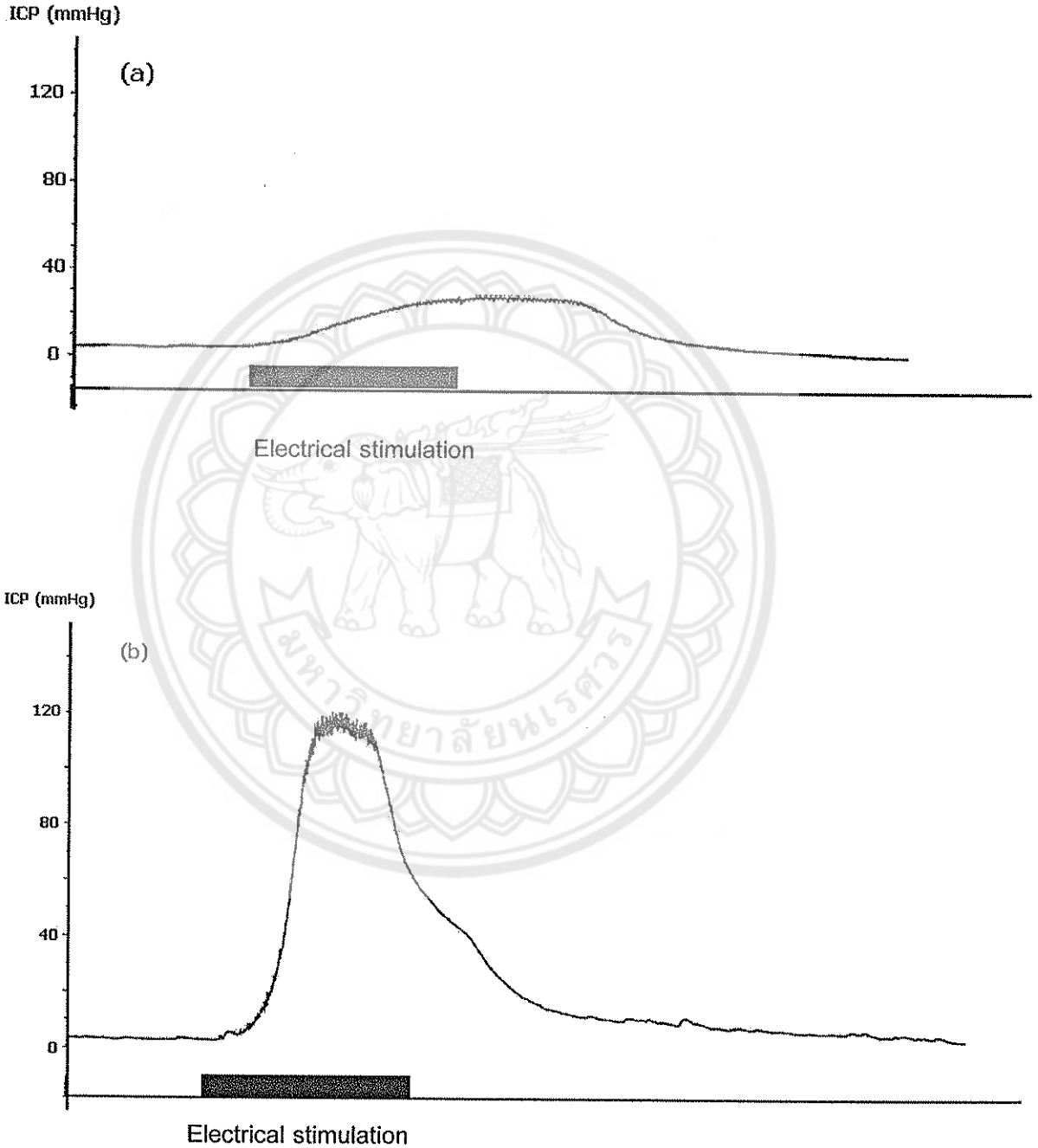


Figure 10 Representative changes in the ICP recorded from (a) a control and (b) a 1 mg/kg BW extract-treated rats. Carvernous nerve stimulus parameters were 5 volts, frequency of 20 Hertz and duration of 5 milliseconds.

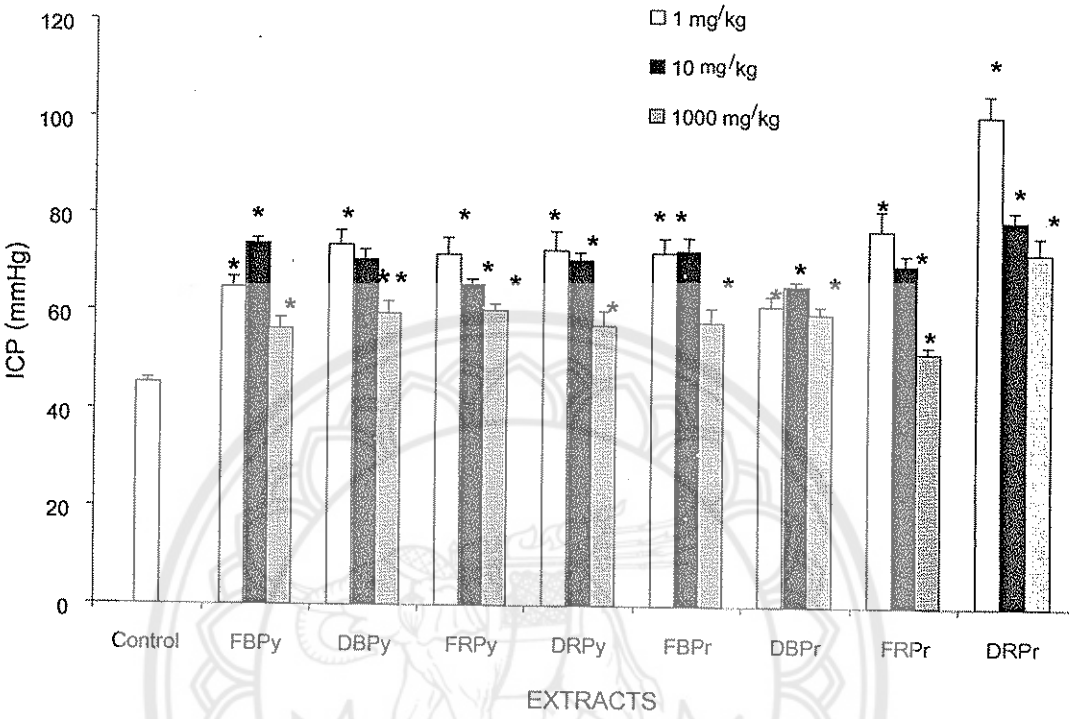


Figure 11 Effects of the extracts from different parts of *B. superba* on the increase ICP. Data are expressed as means ± SE (n=10). FB = fresh bark, DB = dried bark, FR = fresh root, DR = dried root, Py = Phayao province and Pr = Phrae province. * P<0.001 compared to the control group.

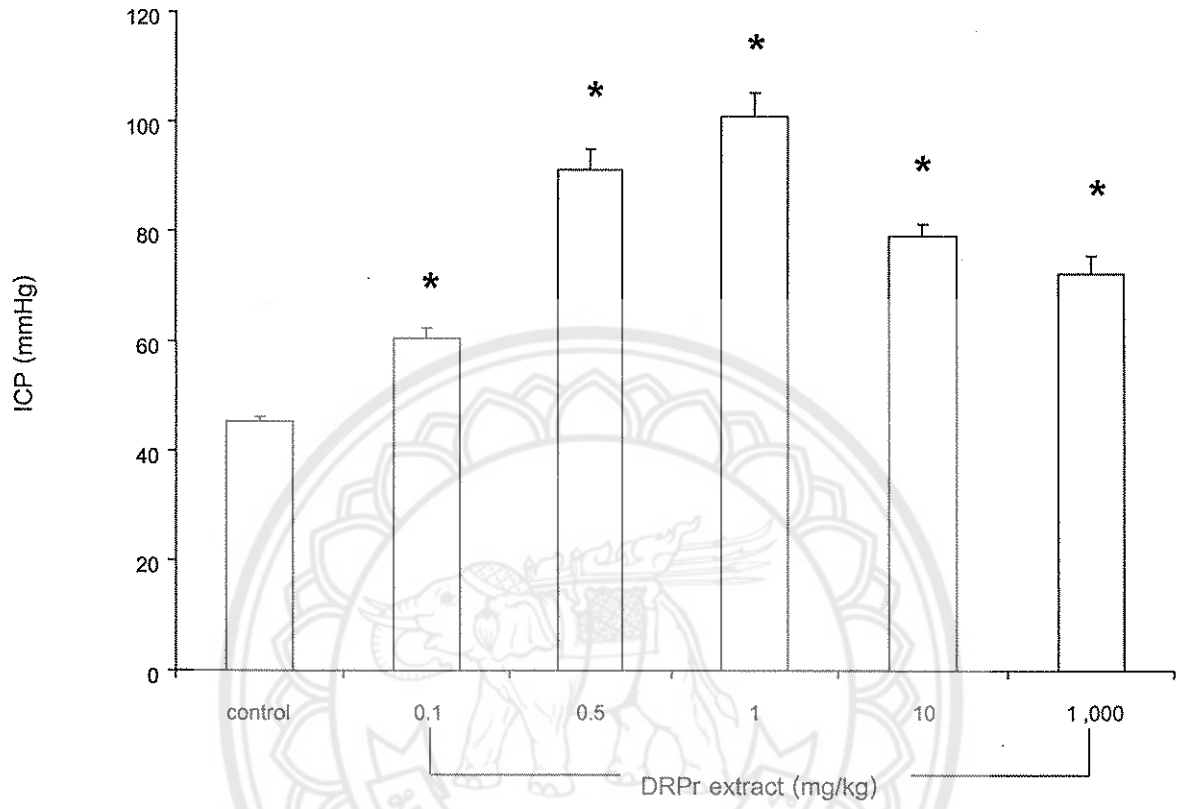


Figure 12 Concentration-response relationship of the *B. superba* extracts from DRPr on the increases in ICP in rats. Data are in means ± SE (n=10). * P<0.0001.

3. Effects of *B. superba* extract on smooth muscle relaxation

Cavernous smooth muscle was removed from rats and placed in an organ bath. The muscle contraction was induced by exposing to phenylephrine (10^{-6} M). Alcoholic extract of *B. superba* dose dependently relaxed phenylephrine-induced smooth muscle contraction (Figure 14). A full relaxation was obtained at the 10 mg/ml of the extract.

IBMX, a non-specific phosphodiesterase inhibitor, and exogenous cGMP also relaxed the cavernous smooth muscle in a dose dependent manner (Figure 15). IBMX at the concentration of 10^{-4} M almost completely relaxed the cavernous smooth muscle. The EC_{50} of IBMX was about $7.2x10^{-6}$ M. cGMP was rather less potent than IBMX. cGMP at the highest concentration studied (10^{-4} M) could induce only about 30% relaxation.

cGMP (10^{-8} – 10^{-4} M) cause the relaxation of phenylephrine (10^{-6} M) – induced contraction of cavernosal strips (Figure 16). The treatment of the strips with the combination of *B. superba* extract at the concentration of 0.01, 0.1 or 1 mg/ml with cGMP (10^{-8}-10^{-4} M) significantly modified the relaxation of cavernosal strips induced by phenylephrine (Figure 16). Addition of *B. superba* extract 0.01 mg/ml enhanced the effect of cGMP with the significant differences at the cGMP concentration 10^{-7} – 10^{-5} M (Figure 16). Whereas the extract at the concentration of 0.1 mg/ml significantly enhanced the effect of cGMP at all concentrations. However, the enhancing effect of 1mg/ml *B. superba* was not significantly observed at the cGMP concentration of 10^{-8} M.

IBMX (10^{-8} – 10^{-4} M) caused the relaxation of pheylephrine–induced contraction of cavernosal strips (Figure 17). The enhancing effects of *B. superba* on IBMX relaxation was not as clear as those observed with cGMP. The extract at the dose of 0.01 mg/ml did not show any enhancing effect of all IBMX concentration studied (Figure 17). The potentiating effect of *B. superba* 0.1 mg/ml was significantly observed when presenting with IBMX only at the concentration of 10^{-5} – 10^{-4} M. Whereas, the extract at the concentration of 1 mg/ml potentiated the IBMX effect at all concentrations studied. The EC50 of the extract at the concentration of 0.01, 0.1 and 1 mg/ml presenting with IBMX are $1.77X10^{-6}$ M, $4.42X10^{-7}$ M and $5.18X10^{-7}$ M, respectively.

4. Effects of *B. superba* extracts on male reproductive system

4.1 Effects on sperm motility

Motility of the sperms from the control rats and mice at time zero were about 90% (Figure 18 and 19). After being removed from cauda epididymis, the ability to move of the sperms gradually declined with time and reached about 70% at 6 hr in both

species. Long-term treatment of the animals with *B. superba* alcoholic extracts at the doses of 0.01, 0.1 or 1.0 mg/BWkg/day for 6 months significantly prolonged the reduced mobility in all animals. After 6 hr the mobility of the sperm from the treated animals still remained at over 75%. However, at the time zero the extract caused no difference in the motility compared to the control group.

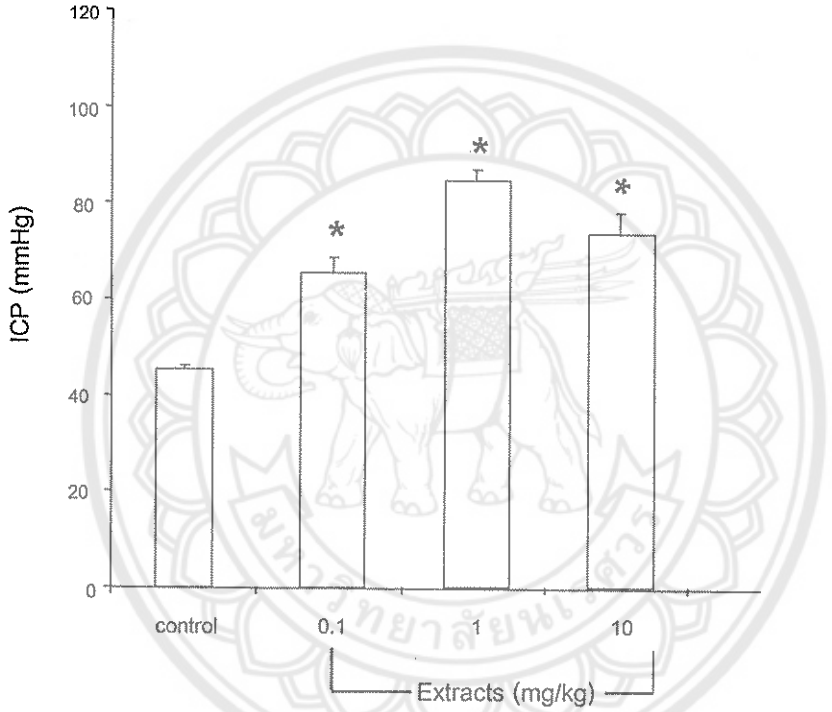


Figure 13 Effect of long-term treatment with *B. superba* extract on the ICP. The animals received the extract daily for 6 months. Data are in means ± SE (n=15).
* P<0.001

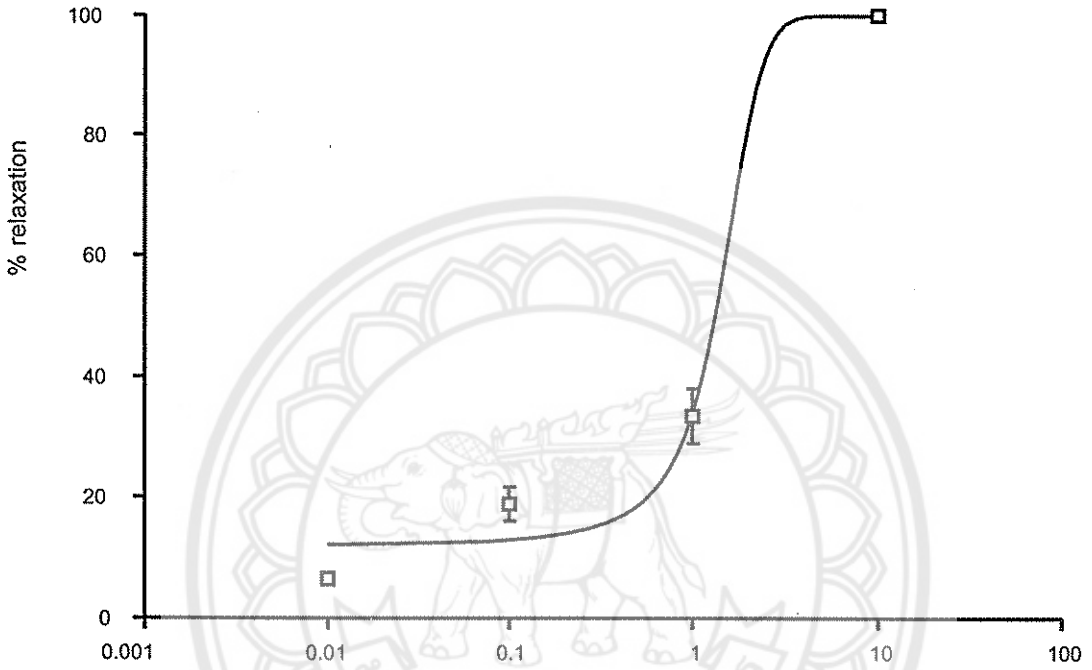


Figure 14 Effects of *B. superba* alcoholic extracts on relaxation of phenylephrine (10^{-6} M) - induced contraction of cavernosal smooth muscle. Data are in means ± SE (n=10).

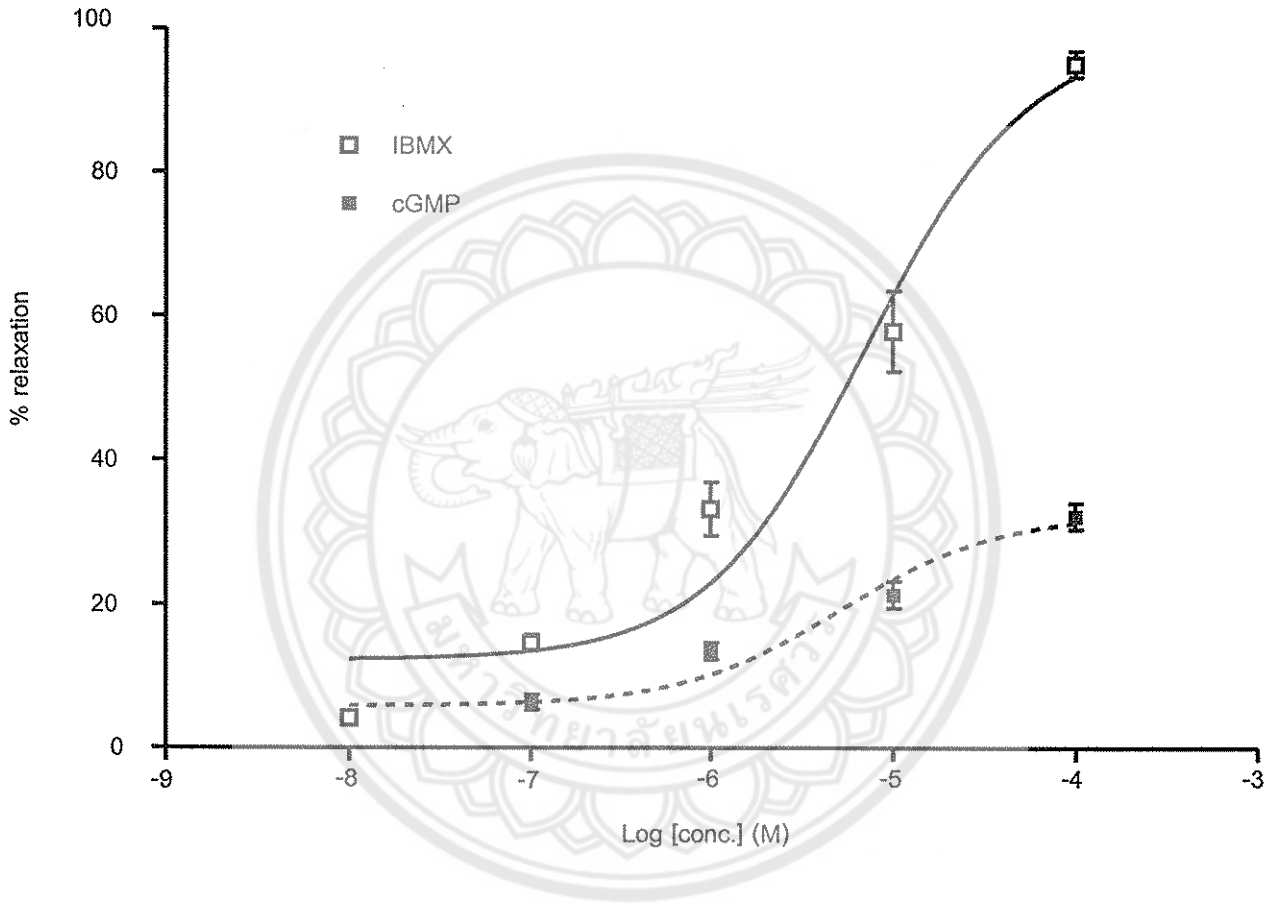


Figure 15 Effects of IBMX and cGMP on relaxation of phenylephrine-induced contraction of cavernosal smooth muscle. Data are in means ± SE (n=10).

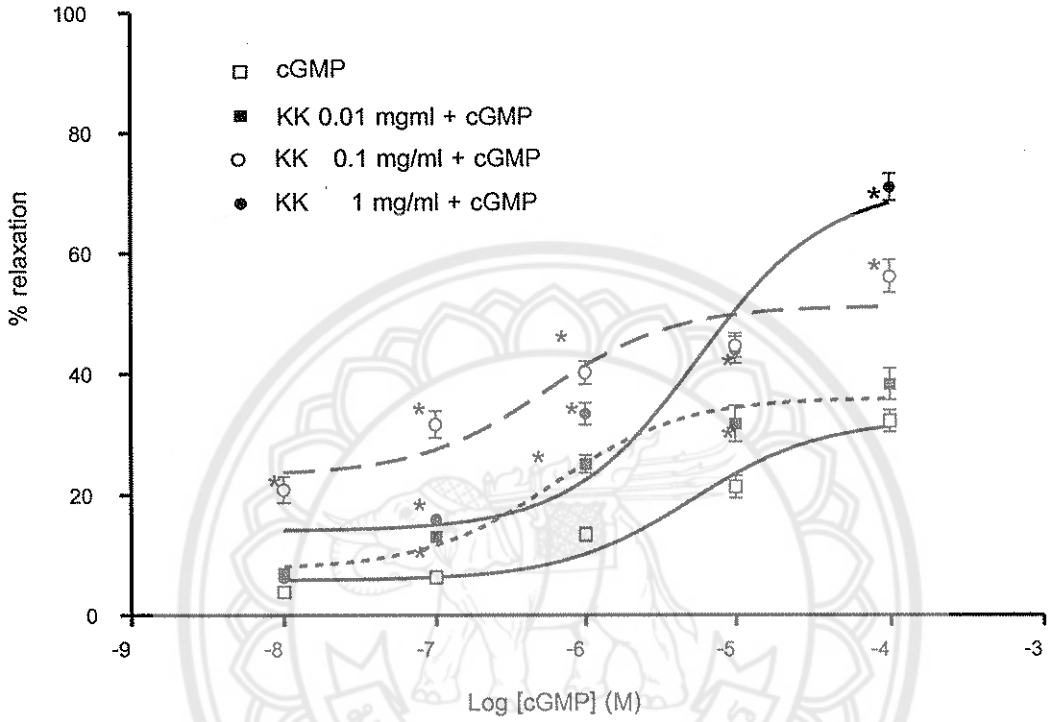


Figure 16 Effects of cGMP or combination of cGMP with various concentrations of *B. superba* extracts (KK) on relaxation of phenylephrine–induced smooth muscle contraction. Data are in means ± SE (n= 10). * P< 0.05.

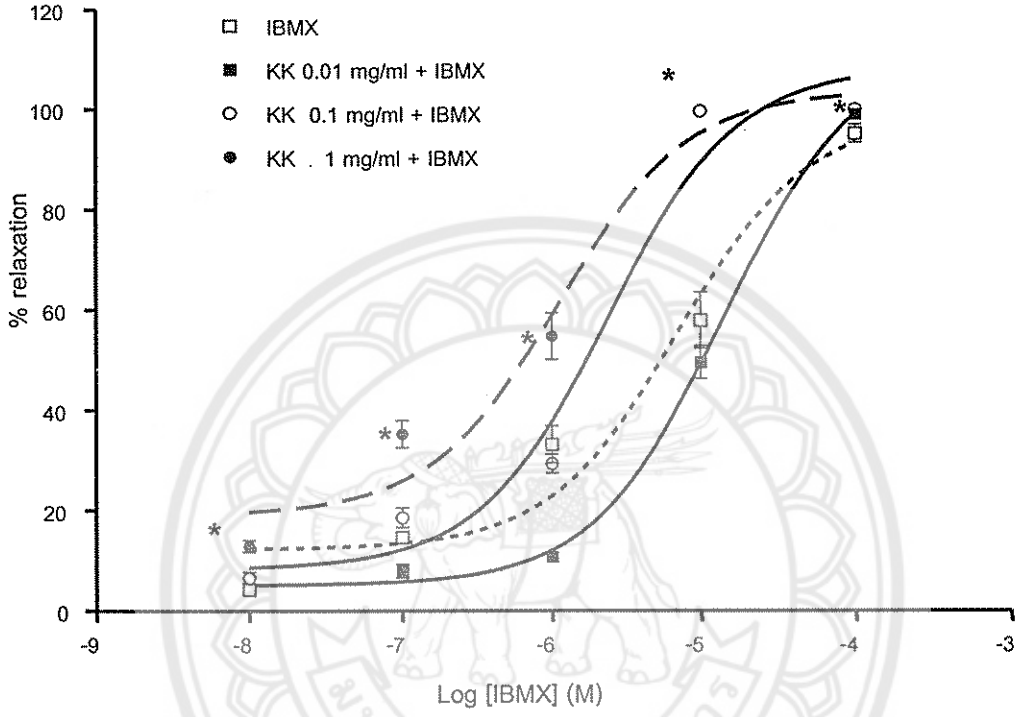


Figure 17 Effects of IBMX or combination of IBMX with various concentrations of *B. superba* extracts (KK) on relaxation of phenylephrine-induced smooth muscle contraction. Data are means ± SE (n=10). * P< 0.05.

4.2 Effects on sperm count

The sperm concentration for normal control rats is about 250 x 10^6/ml as assessed *in vitro* (Figure 20). Long-term exposure (daily for 6 months) of the rats to *B. superba* extracts at the concentration of 0.01, 0.1 or 1 mg/kg BW dose dependently and significantly increased the number of sperm.

Normal control mice possessed smaller sperm concentration than that of the rats. *B. superba* extracts chronically given also dose dependently increased the sperm concentration in mice.

4.3 Effects on sperm morphology

Rats and mice chronically received alcoholic extracts of *B. superba* were examined for sperm and testis morphology. Sperm head and tail anomalies were investigated. The results showed that there was no significant difference in the head and tail anomalies between the extract-treated and the control groups in both rats and mice (Figure 21 and 22, respectively).

Figure 23a and 23b are representative micrographs of testicular sections of a control and a 1 mg/kg extract-treated rats, respectively. The numbers of primary spermatocyte and spermatid of the extract-treated animals were greater than those of the control. However, there was no abnormal pathological sign observed in both the control and the extract-treated animals. In the testicular sections from the treated rats, the germinal epitheliums were well preserved with all the cell types present (Figure 23b). There was no vacuole in the epitheliums and the lamina of the tubules did not contain sloughed immature cell types. There were no signs of interstitial edema, seminiferous tubule degeneration and congestion.

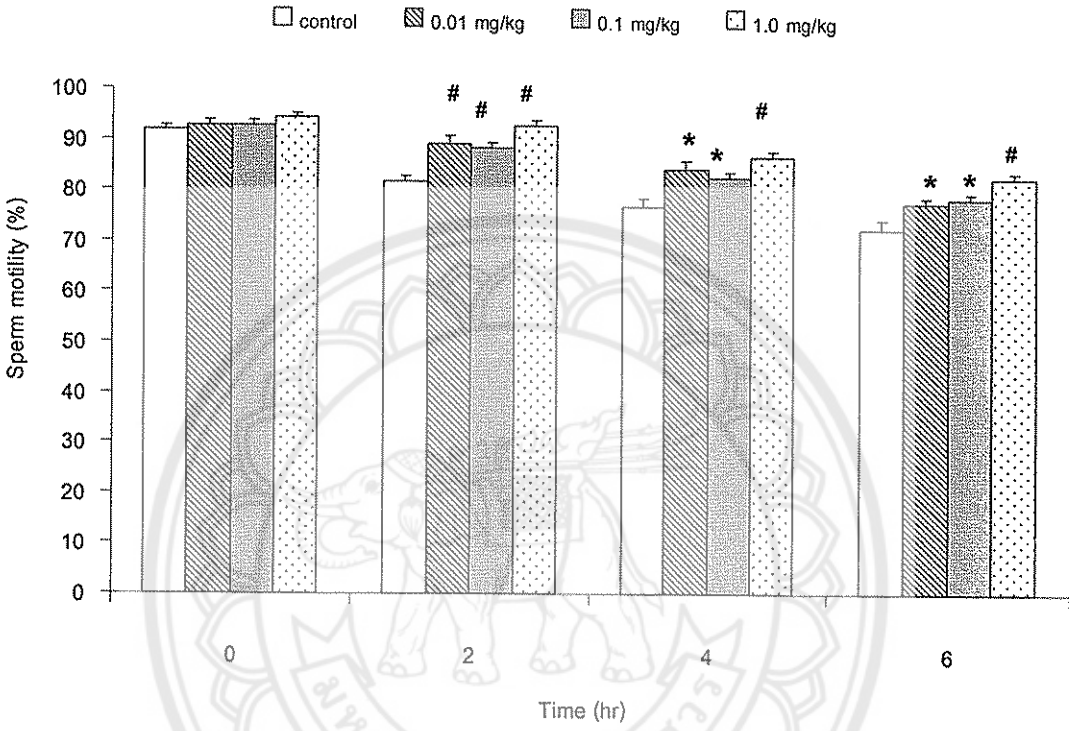


Figure 18 Effects of *B. superba* extracts on the motility of rat spermatozoa. Data are in mean ± SE, n = 6. * P<0.05 and # P<0.005 compared to the control at the same time period.

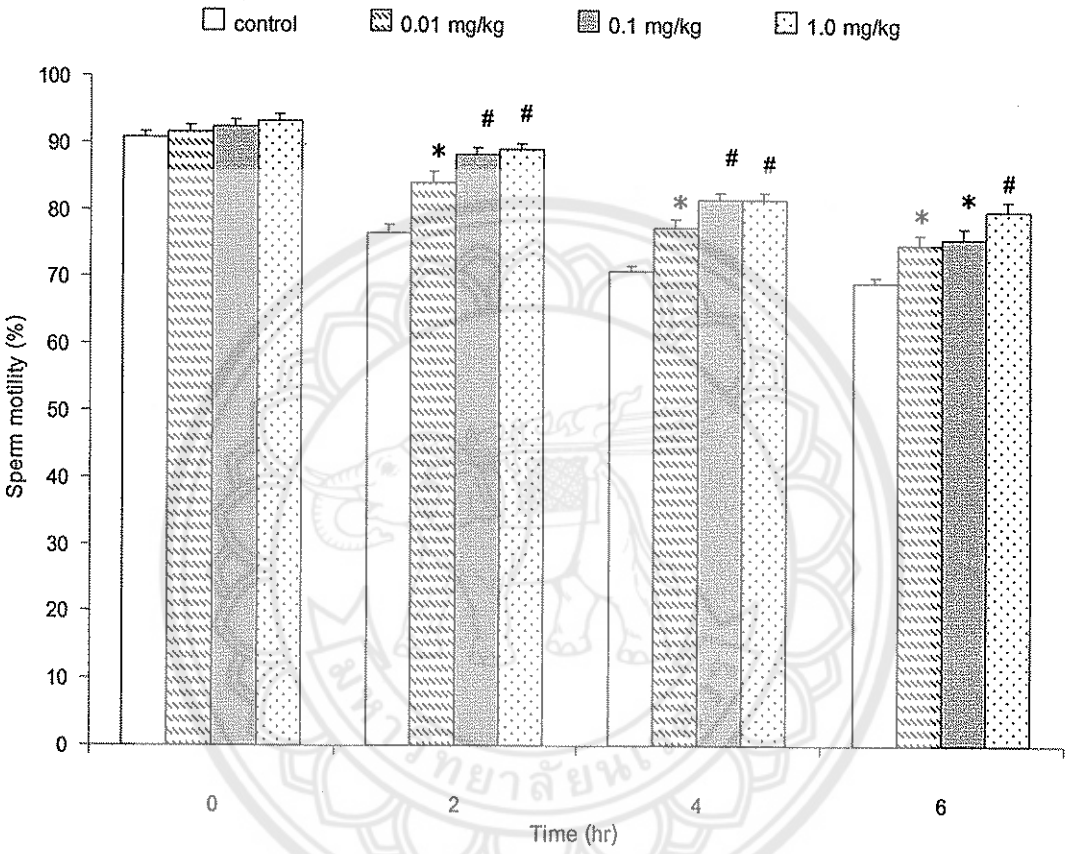


Figure 19 Effects of *B. superba* extracts on the motility of mouse spermatozoa. Data are in means ± SE, n = 6. * P<0.005 and # P<0.0001 compared to the control at the same time period.

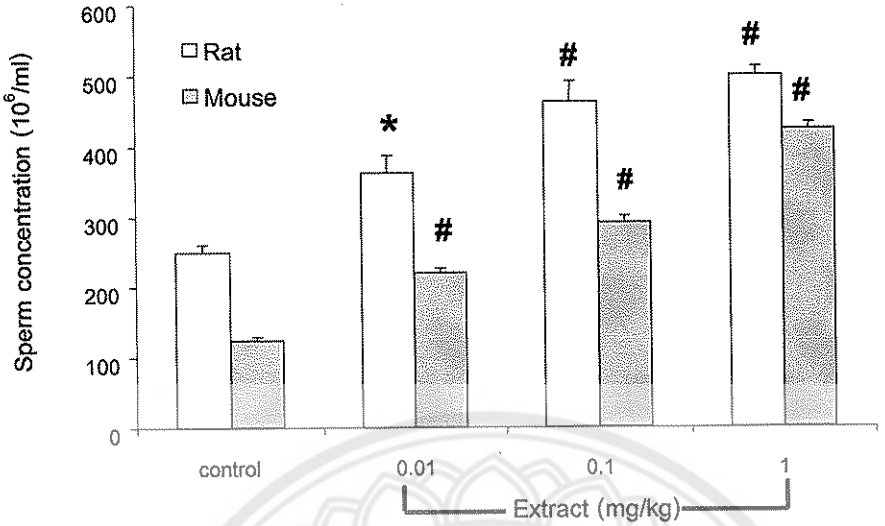


Figure 20 Effects of *B. superba* extracts on the sperm count in rat and mouse. Data are in means ± SE. * P<0.005 and # P<0.0001 compared to their respective control.

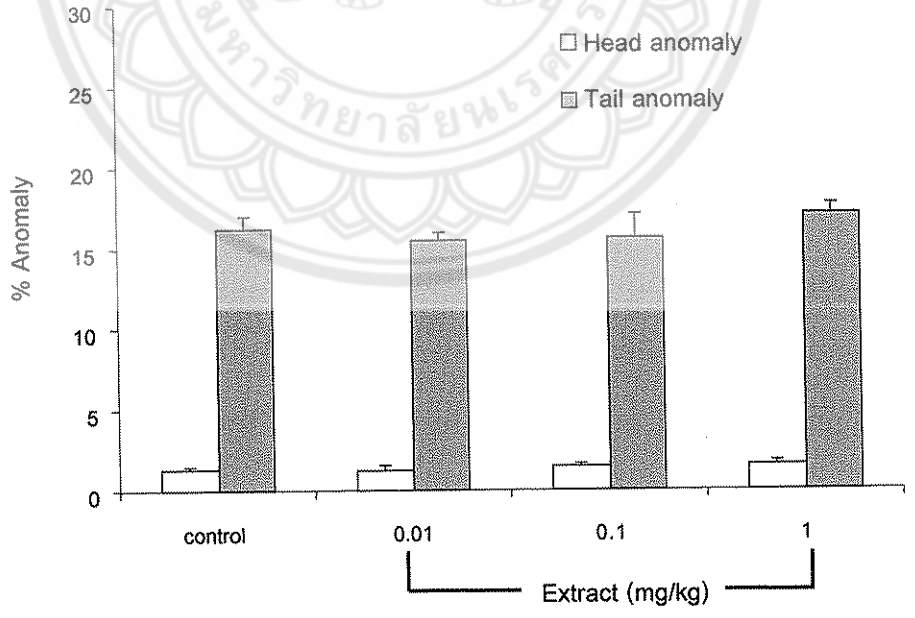


Figure 21 Effects of *B. superba* extracts on the sperm morphology in rats. Data are in means ± SE.

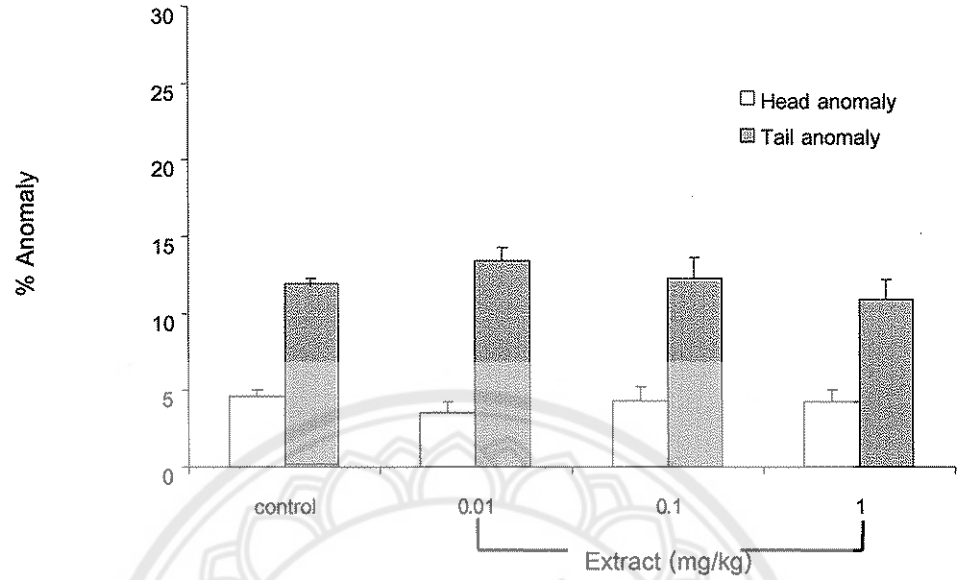


Figure 22 Effects of *B. superba* extracts on the sperm morphology in mice. Data are in means ± SE.

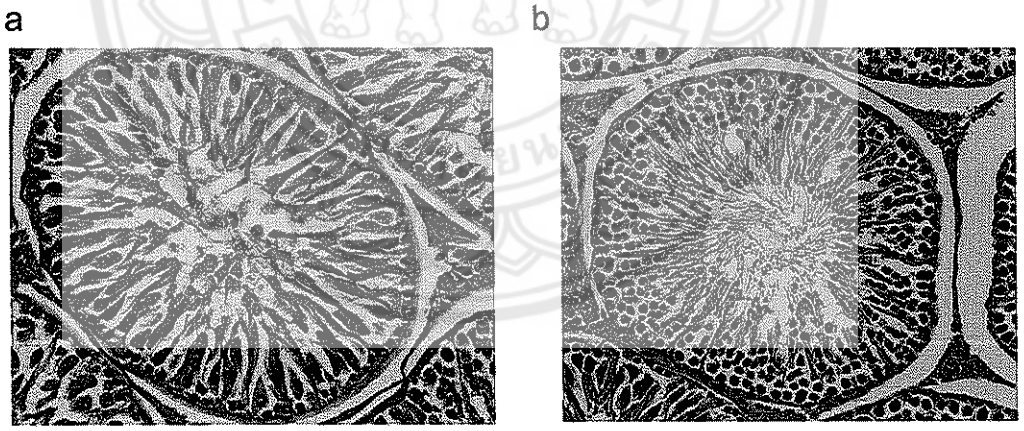


Figure 23 Micrographs of testicular sections from (a) a normal rat and (b) a rat receiving the *B. superba* extracts at the dose of 1 mg/kg BW/day (H&E staining 40X).

Section 2 Toxicity study

1. Acute toxicity study of *B. superba* extracts

1.1 Effects on mortality

In acute toxicity study, female and male rats and mice were administered DW or alcoholic extract of DRPr at the dose of 5,000 mg/kg BW as a single dose. None of the animals died during the first 24 hr after vehicle or extract treatments. At the end of 14-day follow up, none of the mortality was observed. In addition, there were no abnormal signs and symptoms observed. According to the OECD guideline, the LD_{50} of *B. superba* extract is reported as greater than 5,000 mg/kg BW since there was no mortality observed at this treatment dose.

1.2 Effects on organ histology

At the end of 14 days after single treatment the survived animals were sacrificed and organ slides sections were prepared for histological examination. The results revealed no abnormal appearances in almost all animals except only in one female rat whose subendometrial gland was identified as hyperplasia (Table 1, 2).

2. Chronic toxicity study of *B.superba* extracts

2.1 Effects on relative organ weight

Treatment with DRPr for 6 months did not alter organ weights in male rats and female mice (Table 3 and 6, respectively). In female rats, treatment with the extracts also had no effect on the organ weights except the ovary (Table 4). The highest dose (10 mg/kg) of *B. superba* extracts significantly decreased the ovary weight compared to the control group ($P<0.05$). In male mice, alterations in the organ weights were observed in the heart and the lung. The extracts at the concentration of 1 mg/kg BW significantly reduced the heart weight whereas the lower dose (0.1 mg/kg) increased the lung weight (Table 5).

2.2 Effects on hematological parameters

In male rats, *B. superba* extracts at all doses studied had no significant effects on the WBC parameters (Table 7). The extracts at the doses of 1.0 and 10 mg/kg significantly increased the levels of RBC, hemoglobin and hematocrit. The number of platelets was increased in the group treated with 10 mg/kg extract.

In female rats, the treatment with the extracts had no effects on most parameters except on the amounts of neutrophil and lymphocyte, which were significantly decreasesd and increased by the dose of 0.1 mg/kg, respectively (Table 8).

However, no change in the parameters was observed in male and female mice treated with all doses of the extracts (Table 9, 10).

2.2.1 Effects on blood biochemistry

Analysis of blood biochemistry parameters of rats and mice received 0.1, 1 or 10 mg/kg BW *B. superba* extracts for 6 months revealed no significant differences between the treatment groups and the control groups. The functions of the kidney (as reflected by creatinine and uric acid values), and the liver (as reflected by AST, ALT and ALP levels) and cholesterol values were not affected (Table 11-14).

2.3 Effects on organ histology

Chronic administration of *B. superba* extracts caused no toxic effects on the histology of internal organs except in male rats in which 4 from 15 rats treated with 10 mg/kg of the extract showed dilated lumen of epididymidis (Table 15-18). Hepatocyte megalocytosis (Figure 24) was found in male and female mice treated with the extracts at the dose of 10 mg/kg. Figure 25 shows a dilated lumen of epididymidis identified in male rats and mice treated with 10 mg/kg of the extract.

Table 1 Histological results of male rats and mice treated with *B. superba* alcoholic extracts at the dose of 5,000 mg/kg BW as a single dose.

Organs	Lesions	Rats (NA/TN)		Mice (NA/TN)	
		Control (n = 7)	Extract (n = 8)	Control (n = 7)	Extract (n = 8)
Heart	Focal myocardiosis	0	0	0	0
Lung	Lymphoid proliferated peribronchioles	0	0	0	0
Liver	Fatty degeneration	0	0	0	0
	Hepatocyte megalocytosis	0	0	1/7	0
	Lymphoid aggregated periportal area	0	0	0	0
	Bile duct proliferation	0	0	0	0
	Peliosis hepatitis	0	0	0	0
Kidney	Multifocal tubular cyst	0	0	0	0
	Tubular cast	0	0	0	0
	Tubulonephrosis	0	0	0	0
Adrenal gland	Cortical fatty degeneration	0	0	0	0
Testis	Interstitial edema	0	0	0	0
	Seminiferous tubule degeneration	0	0	0	0
	Congestion	0	0	0	0
Epididymis	Dilated lumen	0	0	0	0
Seminal vesicle	Epithelial hyperplasia	0	0	0	0
	Dilated lumen	0	0	0	0

NA/TN = the number of animals with pathological abnormalities / the total number of animals examined

Table 2 Histological results of female rats and mice treated with *B. superba* alcoholic extracts at the dose of 5,000 mg/kg BW as a single dose.

Organs	Lesions	Rats (NA/TN)		Mice (NA/TN)	
		Control (n = 7)	Extract (n = 8)	Control (n = 7)	Extract (n = 8)
Heart	Focal myocardiosis	0	0	0	0
Lung	Lymphoid proliferated peribronchioles	0	0	0	0
Liver	Fatty degeneration	0	0	0	0
	Hepatocyte megalocytosis	0	0	0	0
	Lymphoid aggregated periportal area	0	0	0	0
	Bile duct proliferation	0	0	0	0
	Peliosis hepatitis	0	0	0	0
Kidney	Multifocal tubular cyst	0	0	0	0
	Tubular cast	0	0	0	0
	Tubulonephrosis	0	0	0	0
Adrenal gland	Cortical fatty degeneration	0	0	0	0
Uterus	Subendometrial gland hyperplasia	0	1/8	0	0
Ovary	Ovarian degeneration	0	0	0	0

NA/TN = the number of animals with pathological abnormalities / the total number of animals examined

Table 3 Effects of chronic treatment with *B. superba* extracts on relative organ weights in male rats.

Organs	Control (n = 15)	Extract (mg/kg BW/ day)		
		0.1 (n = 15)	1 (n = 15)	10 (n = 15)
Pituitary gland	0.02 ± 0.002	0.03 ± 0.04	0.02 ± 0.004	0.02 ± 0.003
Brain	4.34 ± 0.34	4.19 ± 0.25	4.33 ± 0.19	4.13 ± 0.35
Heart	2.92 ± 0.21	2.84 ± 0.18	2.88 ± 0.17	2.89 ± 0.14
Lung	3.13 ± 0.20	3.04 ± 0.24	3.15 ± 0.18	3.16 ± 0.24
Liver	26.00 ± 2.29	24.83 ± 1.33	25.27 ± 1.85	25.34 ± 1.49
Kidney	6.06 ± 0.40	5.82 ± 0.32	6.01 ± 0.52	5.91 ± 0.39
Adrenal gland	0.13 ± 0.02	0.12 ± 0.01	0.13 ± 0.02	0.12 ± 0.02
Spleen	1.95 ± 0.16	1.86 ± 0.17	1.89 ± 0.15	1.75 ± 0.07
Prostate gland	0.99 ± 0.21	1.07 ± 0.30	1.14 ± 0.23	0.96 ± 0.21
Seminal vesicle	2.74 ± 0.63	2.61 ± 0.61	2.70 ± 0.59	2.56 ± 0.26
Epididymis	2.64 ± 0.24	2.63 ± 0.23	2.67 ± 0.23	2.62 ± 0.34
Testis	8.94 ± 0.94	8.59 ± 0.73	9.09 ± 0.77	8.82 ± 0.77
Penis	0.65 ± 0.08	0.67 ± 0.09	0.67 ± 0.10	0.67 ± 0.08

Data are in means ± SD

Table 4 Effects of chronic treatment with *B. superba* extracts on relative organ weights in female rats.

Organs	Control (n = 15)	Extract (mg/kg BW/ day)		
		0.1 (n = 15)	1 (n = 15)	10 (n = 15)
Pituitary gland	0.05 ± 0.01	0.05 ± 0.01	0.05 ± 0.01	0.05 ± 0.01
Brain	6.51 ± 0.35	6.53 ± 0.64	6.68 ± 0.85	6.70 ± 0.39
Heart	3.20 ± 0.23	3.14 ± 0.35	3.20 ± 0.23	3.11 ± 0.25
Lung	4.20 ± 0.30	4.12 ± 1.02	4.32 ± 0.37	4.40 ± 0.53
Liver	26.08 ± 2.43	27.60 ± 3.95	28.65 ± 3.59	26.77 ± 4.13
Kidney	6.34 ± 0.55	6.51 ± 0.73	6.67 ± 0.46	6.59 ± 0.62
Adrenal gland	0.24 ± 0.02	0.23 ± 0.04	0.24 ± 0.04	0.24 ± 0.03
Spleen	2.35 ± 0.31	2.36 ± 0.40	2.47 ± 0.29	2.55 ± 0.61
Uterus	1.75 ± 0.52	1.74 ± 0.81	2.12 ± 1.00	2.26 ± 1.54
Ovary	0.42 ± 0.06	0.36 ± 0.11	0.33 ± 0.05	0.32 ± 0.08*

Data are in means ± SD, * $p < 0.05$

Table 5 Effects of chronic treatment with *B. superba* extracts on relative organ weights in male mice.

Organs	Control (n = 12)	Extract (mg/kg BW/ day)		
		0.1 (n = 12)	1 (n = 12)	10 (n = 12)
Brain	12.43 ± 1.26	13.11 ± 0.99	13.13 ± 2.82	12.55 ± 1.35
Heart	4.83 ± 0.68	4.33 ± 0.33	4.22 ± 0.33*	4.41 ± 0.42
Lung	6.27 ± 1.18	7.66 ± 1.08*	6.51 ±1.18	6.62 ± 1.10
Liver	48.20 ± 6.97	43.74 ± 4.06	47.34 ± 3.70	47.03 ± 10.18
Kidney	18.31 ± 2.30	18.82 ± 2.72	19.21 ± 1.92	19.67 ± 2.09
Adrenal gland	0.18 ± 0.05	0.16 ± 0.05	0.16 ± 0.03	0.17 ± 0.14
Spleen	4.07 ± 2.89	3.30 ± 0.87	2.98 ± 0.58	3.32 ± 0.66
Prostate gland	0.43 ± 0.17	0.42 ± 0.10	0.45 ± 0.11	0.47 ± 0.20
Seminal vesicle	9.85 ± 3.18	10.83 ± 2.54	9.24 ± 2.03	9.19 ± 2.16
Epididymis	2.51 ± 0.25	2.88 ± 0.33	2.59 ± 0.39	2.49 ± 0.47
Testis	7.21 ± 0.92	7.61 ± 0.99	7.18 ± 0.95	6.55 ± 1.60
Penis	1.26 ± 0.16	1.25 ± 0.16	1.14 ± 0.19	1.15 ± 0.16

Data are in means ± SD, * $p < 0.05$

Table 6 Effects of chronic treatment with *B. superba* extracts on relative organ weights in female mice.

Organs	Control (n = 15)	Extract (mg/kg BW/ day)		
		0.1 (n = 15)	1 (n = 15)	10 (n = 15)
Brain	15.09± 1.53	15.60 ± 1.93	14.58 ± 1.64	15.50 ± 1.67
Heart	3.74 ± 0.32	3.83 ± 0.56	3.58 ± 0.31	3.64 ± 0.32
Lung	7.86 ± 1.68	7.62 ± 1.42	6.52 ± 1.98	7.56 ± 1.20
Liver	38.09 ± 4.87	42.32 ± 10.43	40.76 ± 3.65	43.56 ± 3.70
Kidney	11.32 ± 1.06	11.69 ± 1.44	11.51 ± 1.30	11.53 ± 0.91
Adrenal gland	0.27 ± 0.04	0.31 ± 0.06	0.29 ± 0.06	0.28 ± 0.07
Spleen	4.24 ± 1.85	4.41 ± 4.22	3.05 ± 0.71	4.81 ± 1.83
Uterus	5.10 ± 3.00	6.20 ± 2.46	4.88 ± 1.65	6.75 ± 2.72
Ovary	0.39 ± 0.12	0.43 ± 0.13	0.43 ± 0.09	0.43 ± 0.13

Data are in means ± SD

Table 7 Effects of chronic treatment with *B. superba* extracts on the hematological parameters in male rats.

Parameters	Control (n = 15)	Extract (mg/kg BW/ day)		
		0.1 (n = 15)	1 (n = 15)	10 (n = 15)
white blood cell ($x10^3/mm^3$)	3.10 ± 0.40	2.82 ± 0.48	3.36 ± 0.76	3.12 ± 0.60
neutrophil (%)	20.10 ± 7.11	21.90 ± 7.61	20.10 ± 10.68	22.27 ± 6.82
lymphocyte (%)	75.13 ± 6.80	76.73 ± 7.98	78.53 ± 10.15	76.80 ± 7.11
monocyte (%)	0.60 ± 0.83	0.80 ± 0.94	0.60 ± 0.91	0.60 ± 0.83
eosinophil (%)	0.87 ± 0.99	0.60 ± 0.99	0.80 ± 1.01	0.33 ± 0.49
basophil (%)	0	0	0	0
red blood cell ($x10^6/ mm^3$)	8.15 ± 0.33	8.31 ± 0.30	8.56 ± 0.33*	8.55 ± 0.39*
platelet ($x10^3/ mm^3$)	724.5 ± 55.5	697.7 ± 56.4	726.8 ± 77.8	800.5 ± 85.5*
hemoglobin (%)	14.90 ± 0.57	15.21 ± 0.57	15.69 ± 0.70*	15.53 ± 0.80*
hematocrit (%)	45.00 ± 1.89	46.00 ± 2.07	47.47 ± 1.96*	46.93 ± 2.05*

Data are in means ± SD, * $p < 0.05$

Table 8 Effects of chronic treatment with *B. superba* extracts on the hematological parameters in female rats.

Parameters	Control (n = 8)	Extract (mg/kg BW/ day)		
		0.1 (n = 8)	1 (n = 8)	10 (n = 8)
white blood cell ($x10^3$/ mm^3)	2.10 ± 0.78	2.59 ± 1.64	2.48 ± 0.87	2.18 ± 1.14
neutrophil (%)	17.38 ± 4.53	11.63 ± 2.62*	19.25 ± 4.74	12.13 ± 4.29
lymphocyte (%)	80.88 ± 4.36	86.63 ± 3.11*	78.90 ± 5.22	86.25 ± 3.69
monocyte (%)	0.50 ± 0.53	0.50 ± 0.53	0.38 ± 0.52	0.75 ± 0.71
eosinophil (%)	1.13 ± 0.99	1.00 ± 0.93	1.50 ± 1.41	0.75 ± 1.04
basophil (%)	0	0	0	0
red blood cell ($x10^6$/ mm^3)	6.81 ± 0.64	6.74 ± 0.71	6.90 ± 0.40	6.77 ± 0.23
platelet ($x10^3$/ mm^3)	550.1 ± 108.2	478.3 ± 75.5	413.1 ± 112.1	544.3 ± 108.5
hemoglobin (%)	12.74 ± 1.36	12.69 ± 1.12	12.85 ± 0.40	12.81 ± 0.37
hematocrit (%)	38.25 ± 3.81	37.88 ± 4.02	37.63 ± 4.07	38.00 ± 1.25

Data are in means ± SD, * $p < 0.05$

Table 9 Effects of chronic treatment with *B. superba* extracts on the hematological parameters in male mice.

Parameters	Control (n = 6)	Extract (mg/kg BW/ day)		
		0.1 (n = 6)	1 (n = 6)	10 (n = 6)
white blood cells ($x10^3$/ mm^3)	2.73 ± 1.12	2.47 ± 1.09	3.73 ± 1.23	3.55 ± 0.63
neutrophil (%)	34.5 ± 5.61	27.50 ± 6.28	31.50 ± 5.24	36.83 ± 4.54
lymphocyte (%)	65.33 ± 5.82	72.17 ± 6.01	68.17 ± 5.27	63.00 ± 4.86
monocyte (%)	0.17 ± 0.41	0.17 ± 0.41	0.33 ± 0.52	0.17 ± 0.41
eosinophil (%)	0	0	0	0
basophil (%)	0	0	0	0
red blood cell ($x10^6$/ mm^3)	7.35 ± 0.42	7.79 ± 0.66	7.69 ± 0.36	7.76 ± 0.24
platelet ($x10^3$/ mm^3)	708.8 ± 104.9	765.2 ± 32.6	718.3 ± 122.8	705.2 ± 84.5
hemoglobin (%)	11.43 ± 0.73	11.78 ± 1.03	11.77 ± 0.85	12.28 ± 0.23
hematocrit (%)	37.00 ± 3.03	36.83 ± 3.31	38.33 ± 2.42	38.67 ± 1.21

Data are in means ± SD

Table 10 Effects of chronic treatment with *B. superba* extracts on the hematological parameters in female mice.

Parameters	Control (n = 6)	Extract (mg/kg BW/ day)		
		0.1 (n = 6)	1 (n = 6)	10 (n = 6)
white blood cells ($x10^3$/ mm^3)	2.95 ± 1.04	4.05 ± 1.58	2.25 ± 1.00	2.27 ± 0.89
neutrophil (%)	31.17 ± 8.57	29.17 ± 11.86	25.33 ± 5.01	20.50 ± 9.35
lymphocyte (%)	68.67 ± 8.29	70.83 ± 11.86	74.67 ± 5.01	79.50 ± 9.35
monocyte (%)	0.17 ± 0.41	0	0	0
eosinophil (%)	0	0	0	0
basophil (%)	0	0	0	0
red blood cell ($x10^6$/ mm^3)	7.41 ± 0.88	7.74 ± 0.54	7.64 ± 0.68	8.00 ± 0.51
platelet ($x10^3$/ mm^3)	451.8 ± 58.3	449.8 ± 82.2	448.8 ± 93.1	417.3 ± 93.3
hemoglobin (%)	11.45 ± 1.07	10.93 ± 2.70	12.05 ± 1.11	12.15 ± 0.96
hematocrit (%)	36.67 ± 2.66	37.17 ± 3.66	38.00 ± 3.58	38.83 ± 2.64

Data are in mean ± SD

Table 11 Effects of chronic treatment with *B. superba* extracts on blood biochemistry of male rats.

Parameters	Control (n = 14)	Extract (mg/kg BW/ day)		
		0.1 (n = 14)	1 (n = 14)	10 (n = 14)
ALP (U/l)	56.9 ± 6.4	52.6 ± 5.8	51.7 ± 5.6	54.4 ± 7.3
AST (U/l)	111.6 ± 35.7	122.9 ± 44.2	94.9 ± 17.2	93.9 ± 25.6
ALT (U/l)	90.0 ± 57.4	83.1 ± 52.1	52.4 ± 0.3	62.9 ± 46.2
uric acid (mg/dl)	0.7 ± 0.3	0.7 ± 0.2	0.9 ± 0.3	0.7 ± 0.3
creatinine (mg/dl)	0.5 ± 0.1	0.5 ± 0.1	0.5 ± 0.2	0.5 ± 0.1
cholesterol (mg/dl)	92.3 ± 13.9	97.1 ± 12.8	93.6 ± 7.8	98.4 ± 12.1

Data are in means ± SD

Table 12 Effects of chronic treatment with *B. superba* extracts on blood biochemistry of female rats.

Parameters	Control (n = 8)	Extract (mg/kg BW/ day)		
		0.1 (n = 8)	1 (n = 8)	10 (n = 8)
ALP (U/l)	40.0 ± 6.3	34.4 ± 2.3	34.1 ± 2.9	37.8 ± 8.7
AST (U/l)	104.6 ± 29.0	102.4 ± 22.3	147.0 ± 53.6	169.0 ± 96.2
ALT (U/l)	44.6 ± 12.7	42.0 ± 9.2	40.4 ± 5.2	42.8 ± 10.2
uric acid (mg/dl)	0.9 ± 0.4	0.9 ± 0.6	0.7 ± 0.3	0.6 ± 0.1
creatinine (mg/dl)	0.46 ± 0.1	0.58 ± 0.4	0.41 ± 0.1	0.43 ± 0.1
cholesterol (mg/dl)	86.9 ± 8.1	91.5 ± 11.6	87.6 ± 15.7	82.0 ± 8.8

Data are in means ± SD

Table 13 Effects of chronic treatment with *B. superba* extracts on blood biochemistry of male mice.

Parameters	Control (n = 8)	Extract (mg/kg BW/ day)		
		0.1 (n = 8)	1 (n = 8)	10 (n = 8)
ALP (U/l)	42.88 ± 19.29	50.25 ± 10.32	52.88 ± 12.21	39.38 ± 7.89
AST (U/l)	244.4 ± 62.0	222.4 ± 74.4	222.8 ± 71.6	190.6 ± 67.8
ALT (U/l)	65.63 ± 15.66	75.63 ± 25.64	71.50 ± 23.92	63.75 ± 23.11
uric acid (mg/dl)	1.59 ± 0.47	1.73 ± 0.58	1.25 ± 0.23	1.34 ± 0.31
creatinine (mg/dl)	0.21 ± 0.04	0.18 ± 0.05	0.20 ± 0.08	0.18 ± 0.05
cholesterol (mg/dl)	87.75 ± 31.18	78.63 ± 14.26	97.75 ± 13.97	81.00 ± 12.06

Data are in means ± SD

Table 14 Effects of chronic treatment with *B. superba* extracts on blood biochemistry of female mice.

Parameters	Control (n = 8)	Extract (mg/kg BW/ day)		
		0.1 (n = 8)	1 (n = 8)	10 (n = 8)
ALP (U/l)	69.38 ± 15.22	85.50 ± 17.30	67.38 ± 11.94	60.88 ± 14.67
AST (U/l)	182.9 ± 51.1	233.4 ± 85.8	195.6 ± 50.6	209.8 ± 53.4
ALT (U/l)	54.38 ± 20.23	50.75 ± 19.72	48.38 ± 4.93	54.88 ± 19.30
uric acid (mg/dl)	1.63 ± 0.83	1.19 ± 0.21	1.79 ± 0.43	1.26 ± 0.44
creatinine (mg/dl)	0.19 ± 0.04	0.18 ± 0.05	0.16 ± 0.05	0.19 ± 0.04
cholesterol (mg/dl)	70.00 ± 11.75	68.38 ± 11.87	78.50 ± 12.27	64.50 ± 8.75

Data are in means ± SD

Table 15 Effects of chronic treatment with *B. superba* extracts on organ histology of male rats.

Organs	Lesions	Control (n = 15)	Extract (mg/kgBW/ day) (NA/TN)		
			0.1 (n = 15)	1 (n = 15)	10 (n = 15)
Heart	Focal myocardiosis	0	0	0	0
Lung	Lymphoid proliferated peribronchioles	0	0	1/15	1/15
Liver	Fatty degeneration	0	0	0	0
	Hepatocyte megalocytosis	0	0	0	0
	Lymphoid aggregated periportal area	0	0	0	0
	Bile duct proliferation	0	0	0	0
	Peliosis hepatitis	0	0	0	0
Kidney	Multifocal tubular cyst	0	0	0	0
	Tubular cast	0	0	0	0
	Tubulonephrosis	0	0	0	0
Adrenal gland	Cortical fatty degeneration	0	0	0	0
Testis	Interstitial edema	0	0	0	0
	Seminiferous tubule degeneration	0	0	0	0
	Congestion	0	0	0	0
Epididymidis	Dilated lumen	0	0	0	4/15*
Seminal vesicle	Epithelial hyperplasia	0	0	0	0
	Dilated lumen	0	0	0	2/15

NA/TN = the number of animals with pathological abnormalities / the total number of animals examined, * $p < 0.05$

Table 16 Effects of chronic treatment with *B. superba* extracts on organ histology of female rats.

Organs	Lesions	Control (n = 15)	Extract (mg/kg BW/ day) (NA/TN)		
			0.1 (n = 15)	1 (n = 15)	10 (n = 15)
Heart	Focal myocardiosis	0	0	0	0
Lung	Lymphoid proliferated peribronchioles	1/15	1/15	0	1/15
Liver	Fatty degeneration	0	0	0	0
	Hepatocyte megalocytosis	0	0	0	0
	Lymphoid aggregated periportal area	0	0	0	0
	Bile duct proliferation	0	0	0	0
	Peliosis hepatitis	0	0	0	0
	Multifocal tubular cyst	0	0	0	0
Kidney	Tubular cast	0	0	0	0
	Tubulonephrosis	0	0	0	0
Adrenal gland	Cortical fatty degeneration	0	0	0	0
Uterus	Subendometrial gland hyperplasia	1/15	0	1/15	2/15
Ovary	Ovarian degeneration	0	0	0	0

NA/TN = the number of animals with pathological abnormalities / the total number of animals examined

Table 17 Effects of chronic treatment with *B. superba* extracts on organ histology of male mice.

Organs	Lesions	Control (n = 12)	Extract (mg/kg BW/ day) (NA/TN)		
			0.1 (n = 12)	1 (n = 12)	10 (n = 12)
Heart	Focal myocardiosis	0	0	0	0
Lung	Lymphoid proliferated peribronchioles	1/12	0	1/12	1/12
Liver	Fatty degeneration	0	0	0	0
	Hepatocyte megalocytosis	0	0	0	1/12
	Lymphoid aggregated periportal area	0	0	0	0
	Bile duct proliferation	0	0	0	0
	Peliosis hepatitis	0	0	0	0
Kidney	Multifocal tubular cyst	0	0	0	0
	Tubular cast	0	0	0	0
	Tubulonephrosis	0	0	0	0
Adrenal gland	Cortical fatty degeneration	0	0	0	0
Testis	Interstitial edema	0	0	0	0
	Seminiferous tubule degeneration	0	0	0	1/12
	Congestion	0	0	0	0
Epididymidis	Dilated lumen	0	0	0	1/12
Seminal vesicle	Epithelial hyperplasia	0	0	0	0
	Dilated lumen	0	0	0	1/12

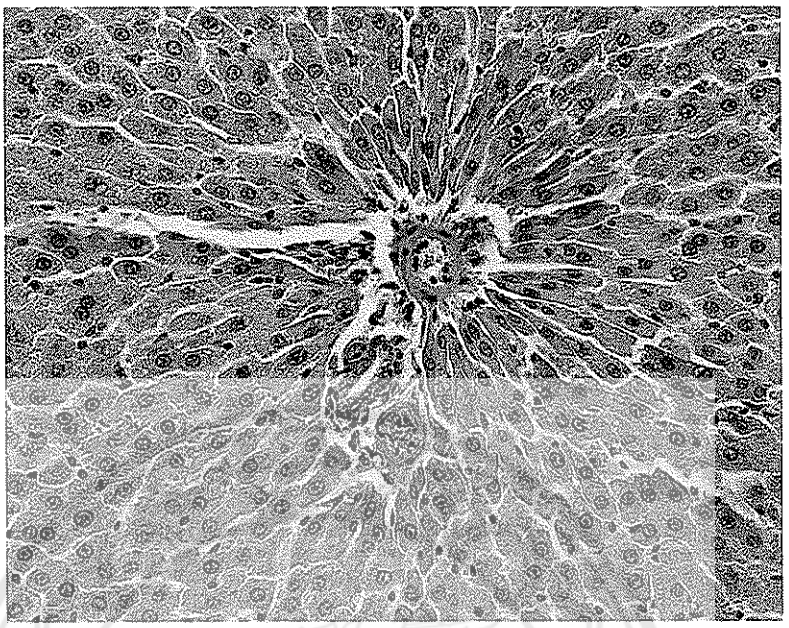
NA/TN = the number of animals with pathological abnormalities / the total number of animals examined

Table 18 Effects of chronic treatment with *B. superba* extracts on organ histology of female mice.

Organs	Lesions	Control (n = 15)	Extract (mg/kg BW/ day) (NA/TN)		
			0.1 (n = 15)	1 (n = 15)	10 (n = 15)
Heart	Focal myocardiosis	0	0	0	0
Lung	Lymphoid proliferated peribronchioles	0	1/15	0	0
Liver	Fatty degeneration	0	0	0	0
	Hepatocyte megalocytosis	0	0	0	1/15
	Lymphoid aggregated periportal area	0	0	0	0
	Bile duct proliferation	0	0	0	0
	Peliosis hepatitis	0	0	0	0
Kidney	Multifocal tubular cyst	0	0	0	0
	Tubular cast	0	0	0	0
	Tubulonephrosis	0	0	0	0
Adrenal gland	Cortical fatty degeneration	0	0	0	0
Uterus	Subendometrial gland hyperplasia	0	0	0	1/15
Ovary	Ovarian degeneration	0	0	0	0

NA/TN = the number of animals with pathological abnormalities / the total number of animals examined

(A)



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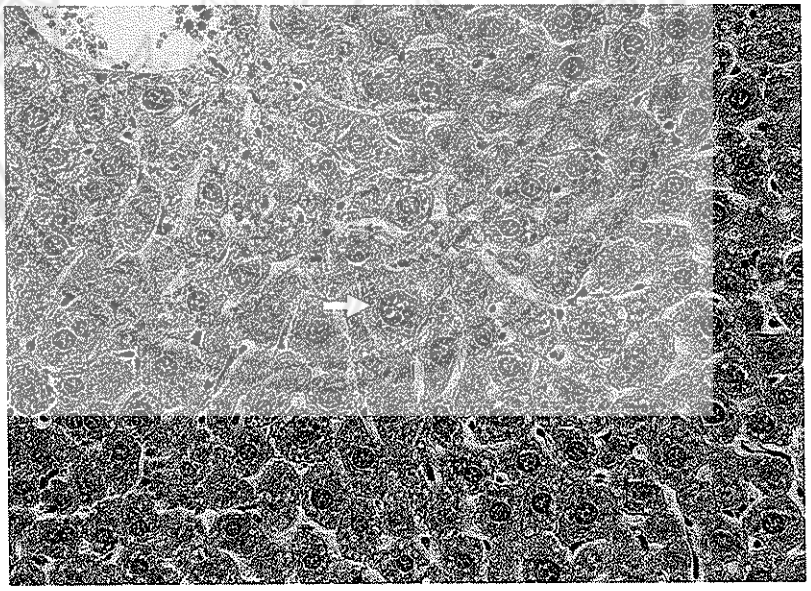
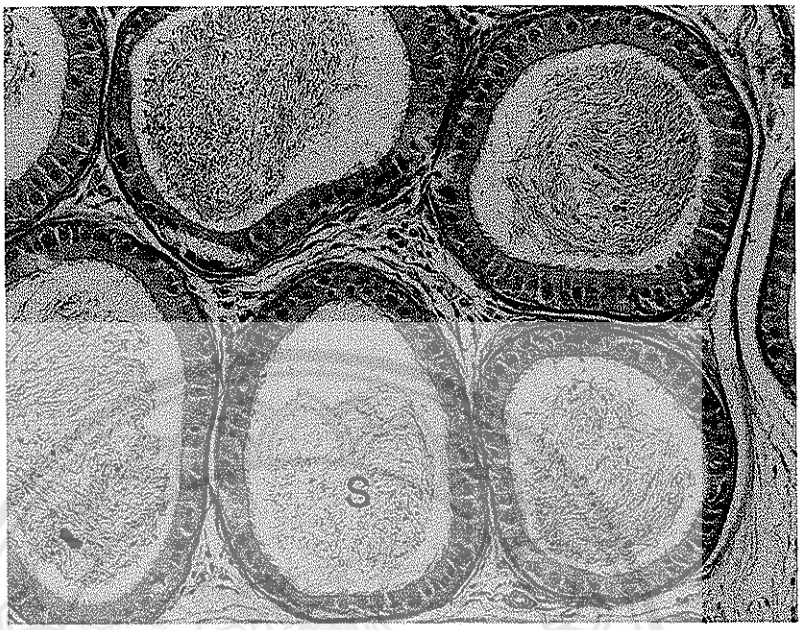


Figure 24 Histological examination of liver sections. (A) from control mouse (B) from one mouse treated with the *B. superba* extract at the dose of 10 mg/kg BW/day. The arrow indicates hepatocyte megalocytosis (H) (H&E staining 40X).

(A)



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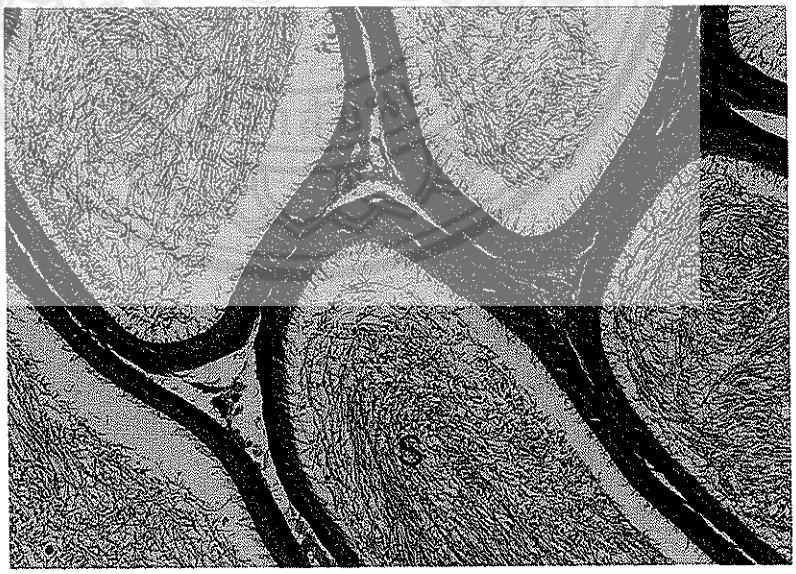

Figure 25 Lumen of epididymidis and spermatozoa (S) in the epididymidis of one (A) control rat and (B) rat receiving *B. superba* extract at the dose of 10 mg/kg BW/day (H&E staining 40X).