CHAPTER IV

RESULTS

1. Effects of *Pueraria mirifica* in high cholesterol-fed rats.

1.1 Physiological and biochemical measurements.

1.1.1 Body weight.

The changes in body weight during the experimental period in three groups of rats are presented in Table 4.1. At the beginning of the study, the body weight of all groups were not significant differences There were a progressive increase in the body weight. Average body weight gain of rats in the cholesterol group was not different from there in control group. However, the body weight gain of rats in cholesterol+*P. mirifica* group was significantly ($P < 0.05$) lower than those in cholesterol group.

1.1.2 Blood biochemistry parameter.

The results of the lipid levels in serum are summarized in Table 4.2. The cholesterol groups showed a considerable rise in serum total cholesterol and low density lipoprotein cholesterol levels compared with control group. The HDL-C/ LDL-C ratio was also significantly lower in the cholesterol group than in the control group ($P< 0.05$). Whereas, total cholesterol, triglyceride, high density lipoprotein cholesterol and low density lipoprotein cholesterol were significantly decreased in cholesterol+*P. mirifica* group compared with those in cholesterol group. In addition, HDL-C/ LDL-C ratio of cholesterol+*P. mirifica* group was not significantly different from control group.

Table 4.1 Changes in the body weight in each group of rats during the experimental dietary period of 12 weeks (g)

Groups	Time period (weeks)						
	0	2	4	6	8	10	12
Control (n= 10)	285.00±17.90	344.20±11.14	375.70±10.54	400.20± 9.28	431.10±10.30	433.80±13.39	461.10± 9.96
Cholesterol (n= 10)	293.90±12.52	351.70± 8.16	391.50± 8.37	417.70± 6.79	449.10± 9.08	465.00± 8.86	472.50±12.81
Cholesterol+*P. mirifica* (n=10)	297.80± 8.50	284.40±13.38*†	317.30±9.09*†	345.90±12.95*†	349.10±10.06*†	366.20±10.18*†	378.70±15.31*†

Data are expressed as mean±S.E.M.

* $P < 0.05$ compared to control group.

† $P < 0.05$ compared to cholesterol group.

Table1. 4.2 **Serum total cholesterol, triglyceride, HDL-C, LDL-C levels and HDL-C/LDL-C ratio in high cholesterol-fed rats.**

Serum lipid parameters / Groups	**Total Cholesterol** (mg / dl)	**Triglyceride** (mg / dl)	**HDL-C** (mg / dl)	**LDL-C** (mg / dl)	**HDL-C/ LDL-C ratio**
Control (n= 10)	64.40±3.18	72.60±7.80	78.67±3.76	8.00±0.50	10.00±0.46
Cholesterol (n= 10)	100.57±8.15*	53.00±4.67	78.57±7.19	71.00±9.19*	1.25±0.20*
Cholesterol+*P. mirifica* (n=10)	39.90±5.05*†	33.50±3.28*†	44.60±8.62*†	16.70±2.79†	7.58±5.44

Data are expressed as mean±S.E.M.

* $P < 0.05$ compared to control group.

† $P < 0.05$ compared to cholesterol group.

1.2 Pharmacological Measurements

1.2.1 Contraction response to noradrenaline.

The concentration response curves to noradrenaline was shown in Fig 4.1. There was not significant differences in neither manimum response nor the sensitivity of aortic vessels between any group, as assessed by comparing the ED_{50} concentrations and maximum response (Table 4.3).

1.2.2 Relaxation response to acetylcholine.

The acetylcholine initiated endothelium-dependent relaxations in aortic rings as shown in Fig 4.2 . The relaxation response of aorta to acetylcholine in control and cholesterol+*P. mirifica* group were significantly greater than that in the cholesterol group. Data were expressed as percentage decreases in relaxation of the aortic ring precontracted by $1x10^{-6}$ M noradrenaline, which averaged 83.31±4.23, 56.14±5.42 and 83.58±2.96 % in the control, cholesterol and cholesterol+ *P. mirifica* group, respectively. In addition, sensitivity of isolated aortic rings to acetylcholine in the cholesterol group was significantly decreased compared with the control group (Table 4.4). Whereas, the cholesterol+*P. mirifica* groups was restored the sensitivity to acetylcholine, as assessed by ED_{50} values of -8.68± 0.57, -7.27±0.13 and -8.39±0.18 logM in the control, cholesterol and cholesterol+*P. mirifica* group, respectively.

1.2.3 Relaxation response to sodium nitroprusside.

Sodium nitroprusside is a NO donor, leading to rise of cGMP-mediated endothelium independent relaxation in smooth muscle cell. There was no significant differences among any groups at any concentration of sodium nitroprusside tested (Fig 4.3-4.4).

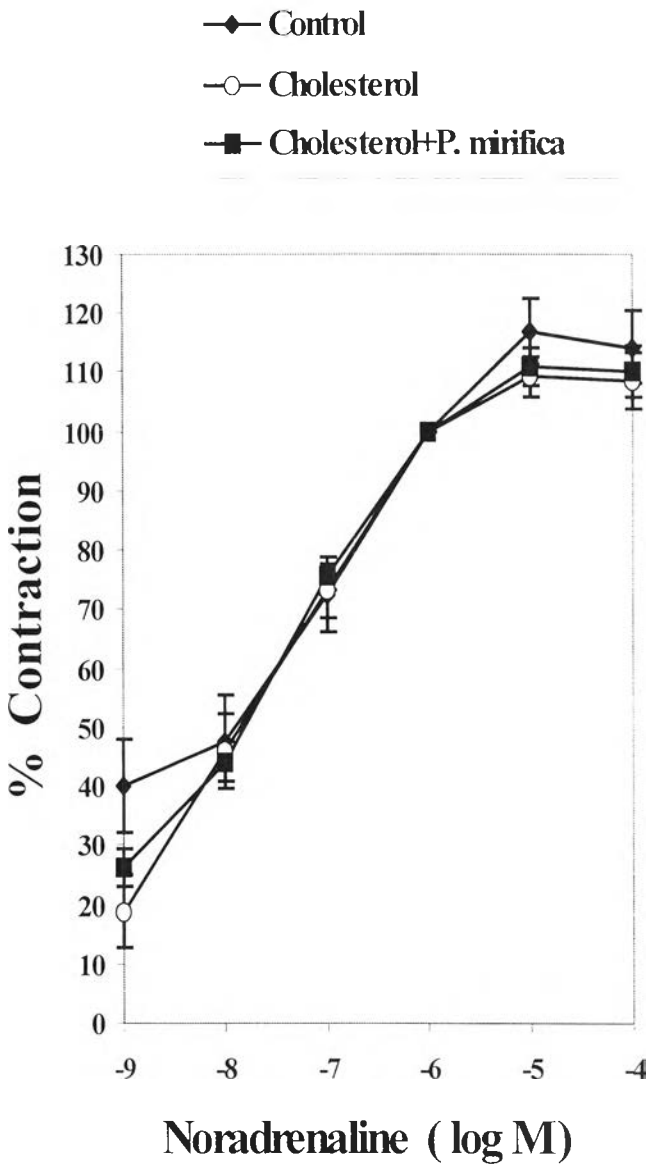

Fig 4.1 Concentration response curve to noradrenaline (NA) of isolated rat aortas from control, cholesterol and cholesterol+*P. mirifica* group (n=10).

Data are express as mean±S.E.M.

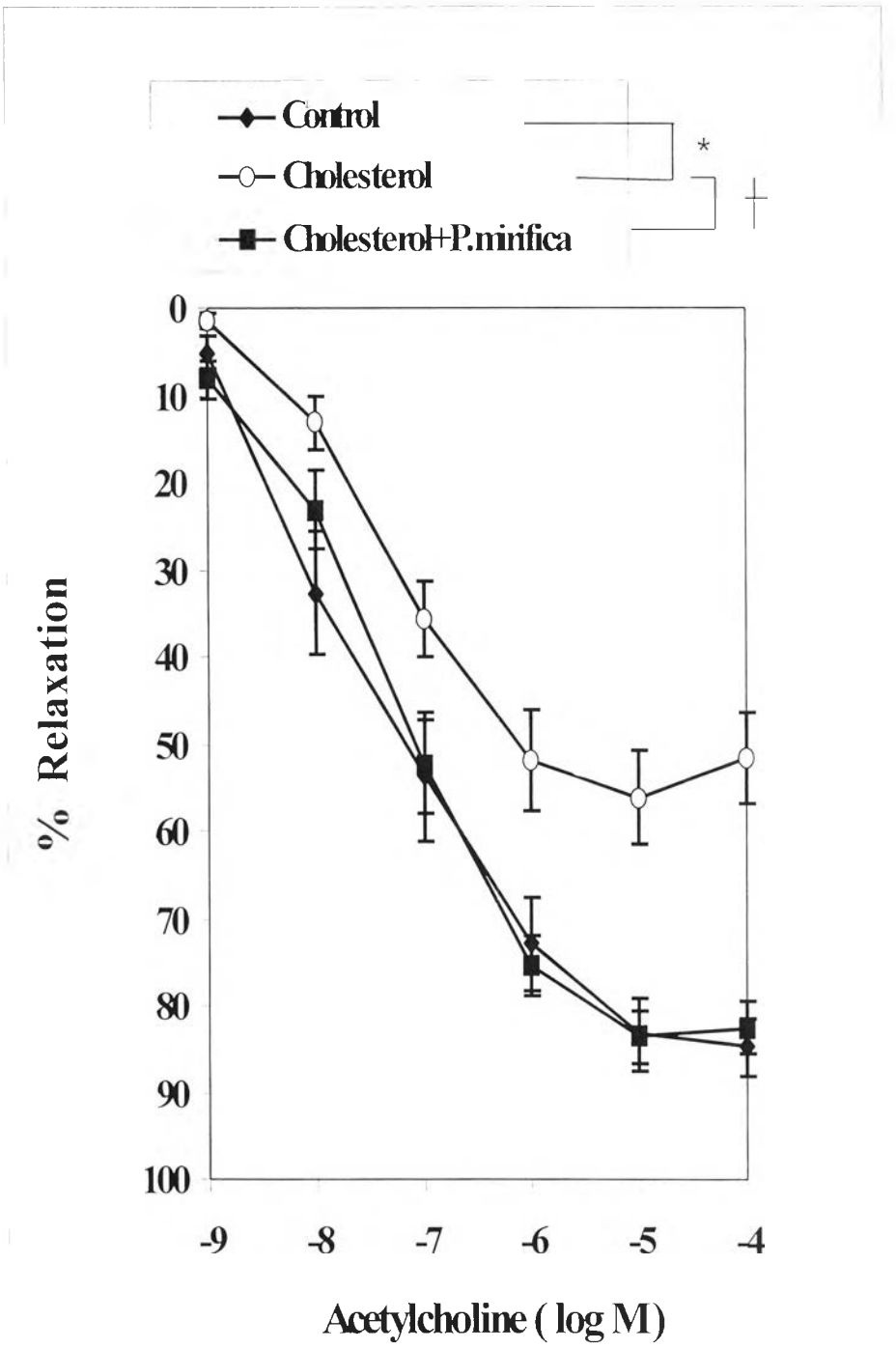

Fig 4.2 Concentration response curves to acetylcholine (Ach) of isolated rat aortas from control, cholesterol and cholesterol+*P. mirifica* groups. Data are shown as mean±S.E.M (n=10).

* $P < 0.05$ compared to control group.

† $P < 0.05$ compared to cholesterol group.

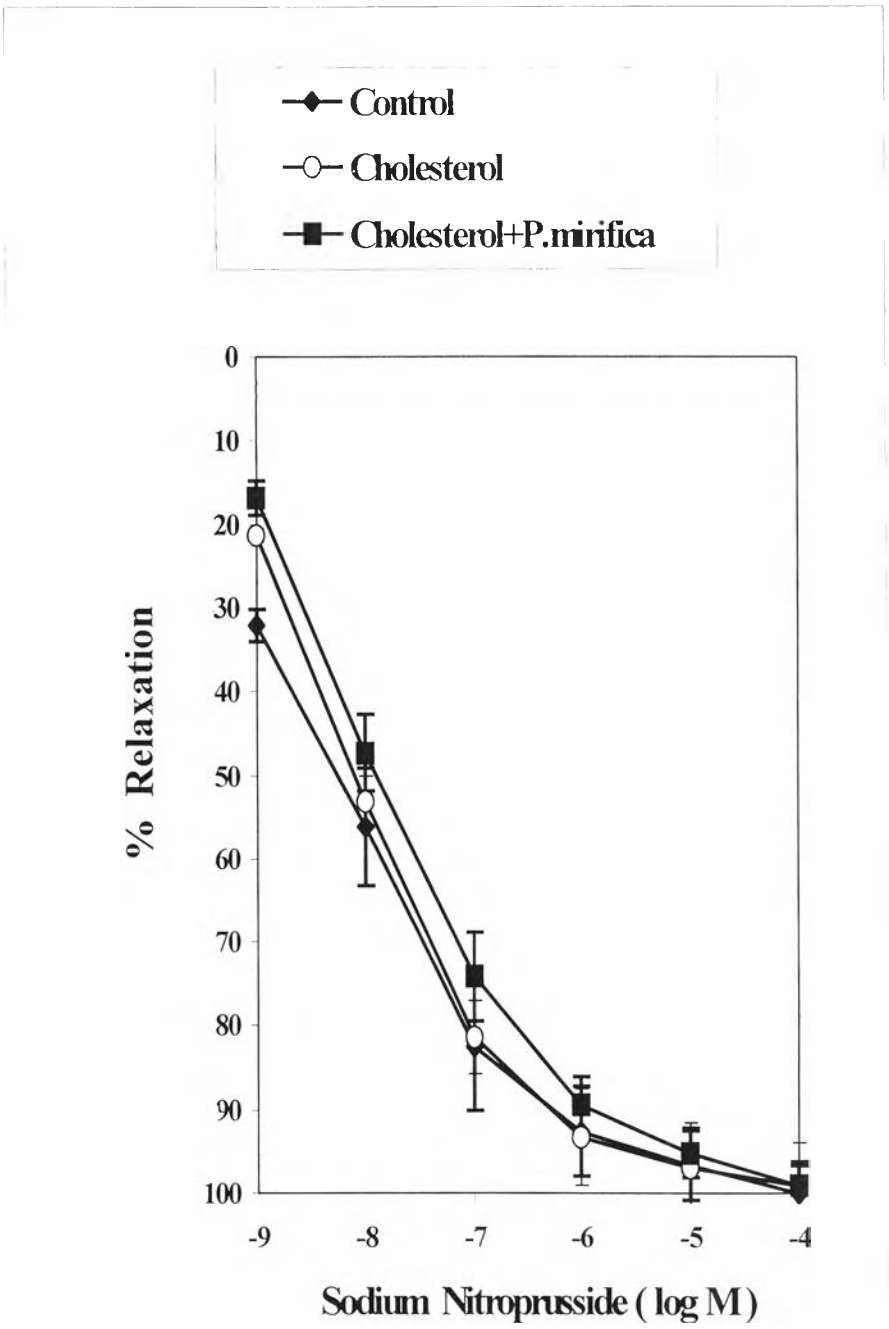

Fig 4.3 Concentration response curves to sodium nitroprusside (SNP) of isolated rat aortas from control, cholesterol and cholesterol+*P. mirifica* groups.

Data are shown as mean±S.E.M (n=10).

* $P < 0.05$ compared to control group.

† $P < 0.05$ compared to cholesterol group

Table4.3 % Maximum response and median effective dose (ED_{50}) of NA in isolated rats aortas.

Groups	% Maximum response	ED_{50} (log M)
Control	117.04±5.49	-8.65±0.19
Cholesterol	109.28±3.33	-8.44±0.15
Cholesterol+*P. mirifica*	111.09±3.16	-8.32±0.07

Data are expressed as mean±S.E.M.

* $P < 0.05$ compared to control group.

† $P < 0.05$ compared to cholesterol group.

Table4.4 % Maximum response and median effective dose (ED_{50}) of Ach and SNP in isolated rats aortas.

Groups	%Maximum response Ach	ED_{50} (log M) Ach
Control	83.31±4.23	-8.03±0.29
Cholesterol	56.14±5.42*	-7.36±0.11*
Cholesterol+*P. mirifica*	83.58±2.96†	-7.90±0.17†

Groups	%Maximum response SNP	ED_{50} (log M) SNP
Control	100.00±0.00	-8.87±0.36
Cholesterol	99.02±0.44	-9.06±0.50
Cholesterol+*P. mirifica*	99.08±0.29	-8.93±0.22

Data are expressed as mean±S.E.M.

* $P < 0.05$ compared to control group.

† $P < 0.05$ compared to cholesterol group.

2 Effects of *Pueraria mirifica* in ovariectomized rabbits.

2.1 Physiological and biochemical measurements.

2.1.1 Body weight.

The body weight gain demonstrated comparability of the four rabbit groups (Table 4.5). At the beginning of the experimental period, the rabbits weights averaged was not significantly different between the groups. The weight gain at the end of 12-week feeding period averaged were 4.23±0.17, 4.17±0.14, 4.22± 0.22 and 4.24±0.13 in the control group, OVX group, OVX+Estrogen group and OVX+*P. mirifica* group, respectively. No significant differences in body weight gain were found between all groups of rabbits.

2.1.2 Blood biochemistry

The serum concentration of total cholesterol, low density lipoprotein cholesterol (LDL-C), high density lipoprotein cholesterol (HDL-C), triglyceride, HDL-C/ LDL-C ratio, direct bilirubin AST, ALT and ALP in all experimental groups of rabbits are presented in Table 4.6 and 4.7. Total serum cholesterol and LDL-cholesterol were 66.05±8.91 and 27.55±6.75 mg/dl, respectively at baseline with no significant differences between these groups. It remained unchanged during the study period in the control, OVX and OVX+Estrogen group but total cholesterol and LDL-cholesterol concentrations slightly decreased on OVX+*P.mirifica* group. Throughout the study, no significant differences in HDL-cholesterol, triglyceride, direct bilirubin, AST, ALT and ALP levels were found between any of these groups.

Table 4.5 Changes in the body weight in each group of rabbits during the experimental dietary period of 12 weeks (kg)

Groups	Time period (weeks)											
	0	2	3	4	5	6	7	8	9	10	11	12
Intact Control	3.59±0.08	3.71±0.06	3.73±0.08	3.78±0.11	3.79±0.11	3.94±0.12	4.10±0.15	4.08±0.13	4.14±0.14	4.15±0.14	4.22±0.16	4.23±0.17
OVX	3.21±0.30	3.64±0.19	3.82±0.13	3.84±0.11	3.90±0.11	3.96±0.13	3.96±0.13	3.99±0.13	3.98±0.14	4.01±0.14	4.10±0.14	4.17±0.14
OVX+Estrogen	3.97±0.11	4.01±0.12	4.03±0.16	4.08±0.17	4.08±0.17	4.21±0.20	4.23±0.21	4.21±0.21	4.18±0.21	4.19±0.24	4.18±0.22	4.22±0.22
OVX+*P. mirifica*	3.59±0.21	3.82±0.14	3.94±0.13	3.97±0.13	4.00±0.14	4.01±0.13	4.10±0.14	4.14±0.13	4.18±0.12	4.19±0.10	4.22±0.13	4.24±0.13

Data are expressed as mean±S.E.M. of samples from 5 animals.

* $P < 0.05$ compared to control group.

Table 4.6 Serum lipid parameter of rabbit (n=5).

Group	Time period (weeks)			
	0	4	8	12
Total-Cholesterol (mg/dl)				
Intact Control	52.20±4.53	58.20±6.66	63.40±6.79	65.80±5.68
OVX	57.80±12.34	51.20±9.43	67.00±5.21	65.00±9.97
OVX+Estrogen	56.20±4.69	55.60±4.09	62.80±3.25	61.40±4.27
OVX+*P. mirifica*	98.00±31.12	60.60±9.03	59.80±10.08	76.40±26.32
HDL-Cholesterol (mg/dl)				
Intact Control	35.90±2.95	40.96±4.64	45.98±4.79	45.48±3.96
OVX	43.00±11.72	43.40±7.05	46.80±5.62	45.70±8.09
OVX+Estrogen	38.60±4.13	38.72±2.50	41.70±2.36	39.62±4.10
OVX+*P. mirifica*	49.20±17.20	46.84±5.24	44.10±7.46	42.56±7.04
LDL-Cholesterol (mg/dl)				
Intact Control	23.40±4.17	17.00±3.49	11.00±1.22	12.80±0.92
OVX	17.20±5.91	12.20±3.80	11.75±2.46	12.20±3.40
OVX+Estrogen	16.40±2.36	11.60±3.33	15.00±2.70	14.60±1.25
OVX+*P. mirifica*	53.20±24.09	12.75±6.47	12.80±4.28	34.20±21.96
HDL/LDL ratio				
Intact Control	1.74±0.34	2.52±0.55	4.20±0.14	3.55±0.16
OVX	5.82±3.37	5.03±1.31	4.61±1.26	4.66±0.83
OVX+Estrogen	2.59±0.46	5.24±2.24	3.17±0.57	2.80±0.38
OVX+*P. mirifica*	3.61±1.47	7.32±5.09	6.16±2.36	3.02±0.84
Triglycerides (mg/dl)				
Intact Control	38.00±7.79	45.80±5.62	55.00±9.68	51.40±3.44
OVX	68.40±12.31	68.20±8.73	76.00±24.42	80.00±23.11
OVX+Estrogen	65.00±18.08	33.60±3.61	45.60±8.91	70.82±14.41
OVX+*P. mirifica*	51.80±9.35	47.80±5.94	50.20±2.58	61.60±2.71

Data are expressed as mean±S.E.M.

* $P < 0.05$ compared to control group.

† $P < 0.05$ compared to OVX group.

Table 4.7 Blood biochemistry of rabbit (n=5).

Group	Time period (weeks)			
	0	4	8	12
ALT (SGPT) (U/I)				
Intact Control	30.60±2.73	33.20±9.12	31.00±4.27	39.20±7.79
OVX	47.00±11.88	38.40±3.12	32.50±5.04	44.60±6.53
OVX+Estrogen	32.40±3.83	31.40±5.41	24.60±4.14	35.80±7.62
OVX+*P. mirifica*	36.80±8.40	43.60±7.98	32.60±5.81	50.60±10.84
ALK.Phosphatase (U/I)				
Intact Control	69.20±9.34	64.60±16.38	52.40±8.07	53.60±12.10
OVX	61.67±6.89	49.40±6.52	48.25±11.05	51.00±10.63
OVX+Estrogen	44.40±5.09	62.56±22.48	48.00±11.30	43.60±8.72
OVX+*P. mirifica*	45.33±9.82	52.00±5.54	43.20±4.04	42.60±4.23
AST (SGOT) (U/I)				
Intact Control	12.20±2.27	16.80±3.76	17.20±1.80	43.80±4.98
OVX	23.40±3.19	26.00±2.17	19.75±2.25	36.40±10.57
OVX+Estrogen	13.20±1.39	21.20±4.04	16.00±5.22	37.00±8.17
OVX+*P. mirifica*	17.80±4.50	21.80±4.76	16.40±3.91	37.60±9.14

Data are expressed as mean±S.E.M.

* $P < 0.05$ compared to control group.

† $P < 0.05$ compared to OVX group.

2.2 Pharmacological Measurements

2.2.1 Contraction response to noradrenaline.

Conentration response curve to noradrenaline of isolated rabbit aortas from every groups were shown in Fig. 4.4. There was no any significant difference in the sensitivity and maximum response between the groups (Table 4.8).

2.2.2 Relaxation response to acetylcholine.

Endothelium-dependent relaxation to acetylcholine are shown in Fig 4.5. After further 12 weeks of ovariectomy, endothelium-dependent relaxations to acetylcholine were severely impaired. The concentration –relaxing response to acetylcholine from the control group was signifiantly greater than that in the OVX group. The relaxation response curves to acetylcholine of isolated aortas from ovariectomized rabbits treated with 17β-estradiol and *P. mirifica* demonstrated a significant improvement in endothelium-dependent vasorelaxation. The maximal relaxation to acetylcholine were about 81±2.26, 41.21±3.23, 75.77±4.54 and 76.21±3.09 % of the response in the control, OVX, OVX+Estrogen and OVX+*P.mirifica* group, respectively. The sensitivity to acetylcholine from the OVX group significantly decreased when compared with the control group, as assessed by ED_{50} values of -7.53±0.10 logM VS -9.61±0.72 log M for the OVX and control group, respectively (Table 4.9). Whereas, the OVX+Estrogen and OVX+*P. mirifica* were restored the sensitivity to acetylcholine.

2.2.3 Relaxation response to sodium nitroprusside.

The concentration-response curve to sodium nitroprusside are presented in Fig 4.6. Endothelium-independent relaxations in response to sodium nitroprusside were not significantly different between all of the groups. The ED_{50} values of sodium nitroprusside were also not significantly different between group (Table 4.9).

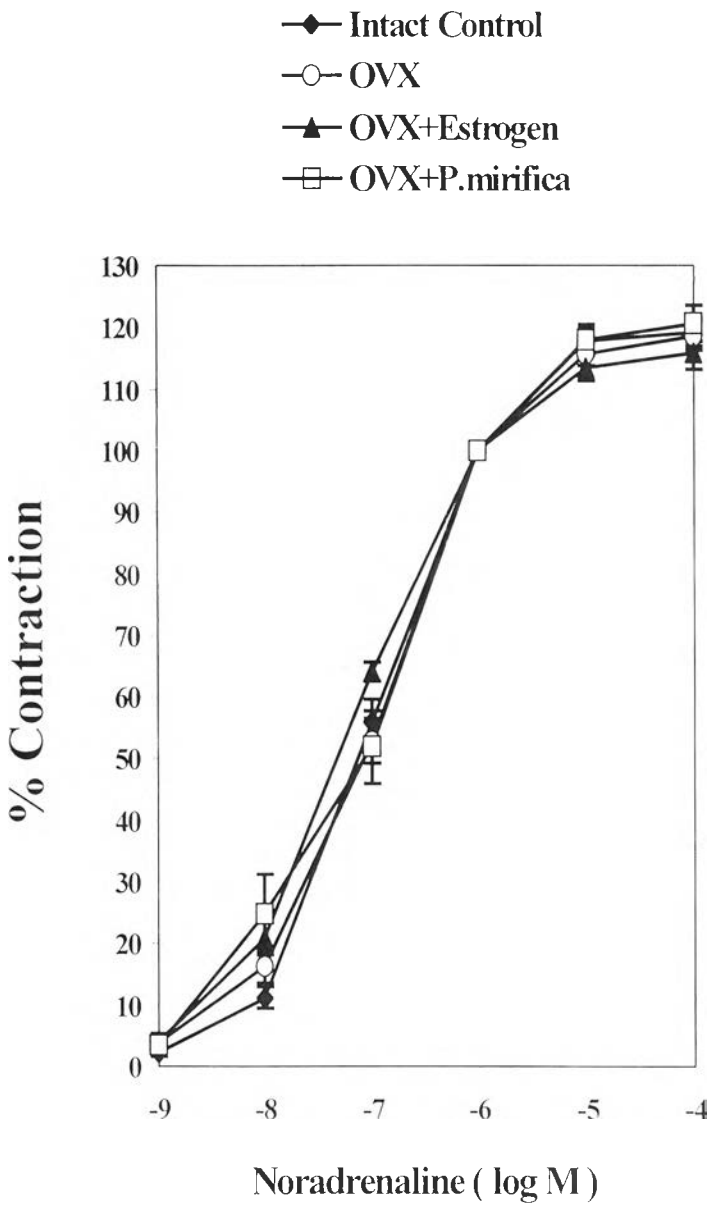

Fig 4.4 Concentration response curve to noradrenaline (NA) of isolated rabbit aortas from intact control, OVX, OVX+Estrogen and OVX+*P. mirifica* groups (n=5).

Data are express as mean± S.E.M.

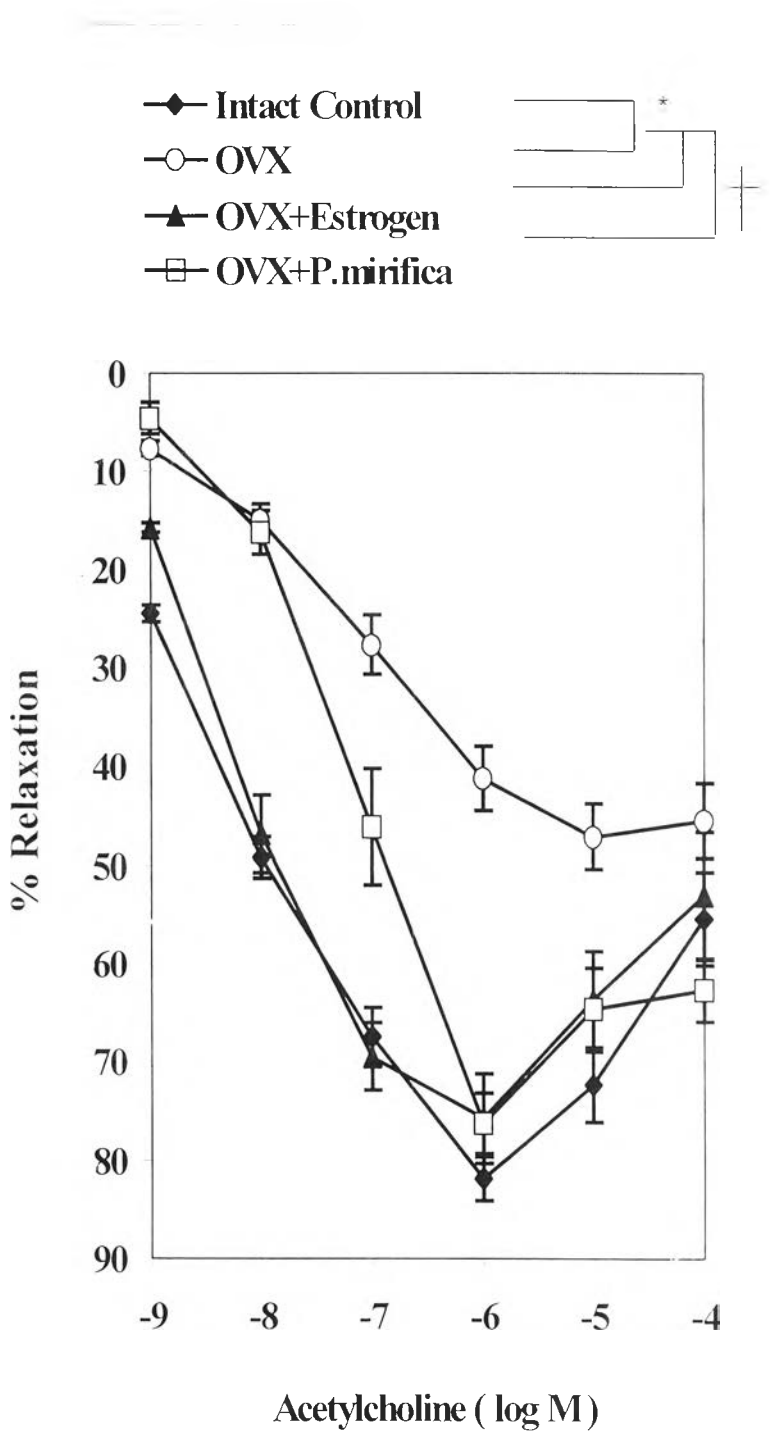

Fig. 4.5 Concentration response curves to acetylcholine (Ach) of isolated rabbit aortas from intact control, OVX, OVX+Estrogen and OVX+*P.mirifica* groups.

Data are shown as mean±S.E.M (n=5).

* $P < 0.05$ compared to control group.

† $P < 0.05$ compared to OVX group.

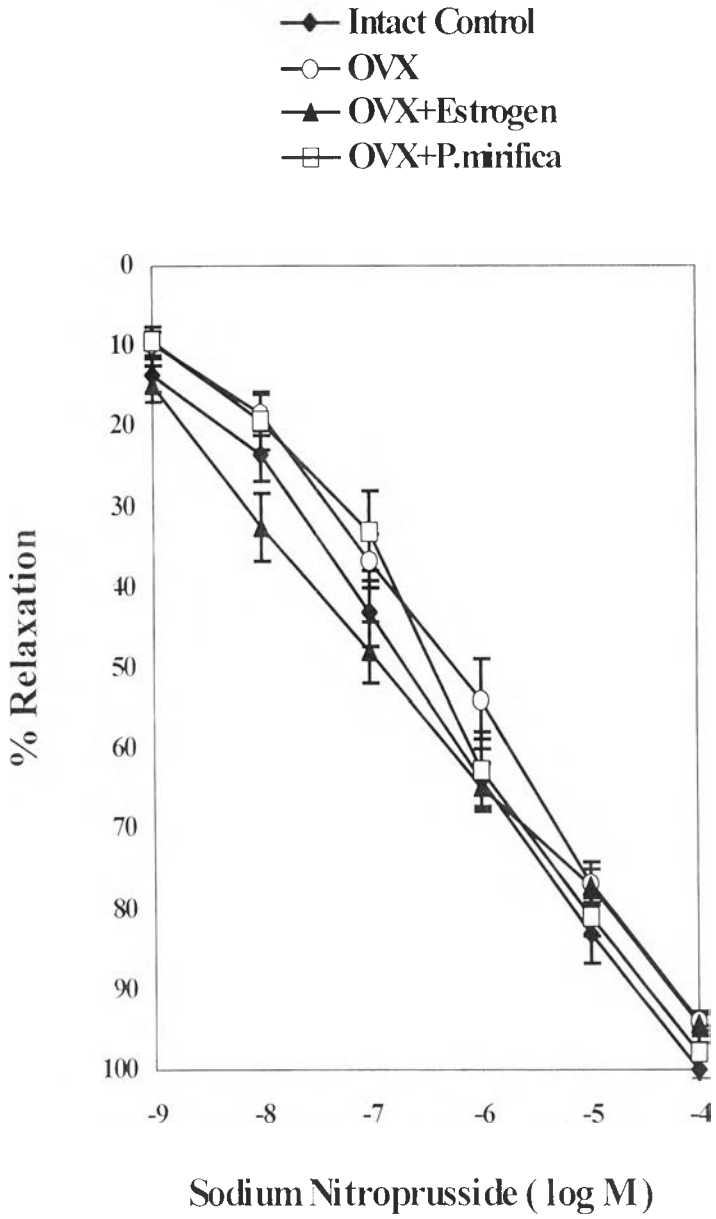

Fig 4.6 Concentration response curves to sodium nitroprusside (SNP) of Isolated rabbit aortas from intact control, OVX, OVX+Estrogen and OVX+*P.mirifica* groups (n=5).

Data are shown as mean±S.E.M.

* $P < 0.05$ compared to control group.

† $P < 0.05$ compared to OVX group.

Table4.8 % Maximum response and median effective dose (ED_{50}) of NA in isolated rabbits aortas.

Groups	%Maximum response	ED_{50} (log M)
Intact Control	119.32±2.33	-7.37±0.04
OVX	118.69±2.38	-7.44±0.05
OVX+ Estrogen	116.01±2.72	-7.53±0.04
OVX+*P. mirifica*	120.62±2.89	-7.50±0.12

Data are expressed as mean±S.E.M.

* $P < 0.05$ compared to control group.

† $P < 0.05$ compared to OVX group.

Table 4.9 % Maximum response and median effective dose (ED_{50}) of Ach and SNP in isolated rabbit aortas.

Groups	%Maximum response Ach	ED_{50} (log M) Ach
Intact Control	81.82±2.26	-9.61±0.72
OVX	41.21±3.24*	-7.53±0.10*
OVX+ Estrogen	75.77±4.54†	-9.53±0.32†
OVX+*P. mirifica*	76.21±3.09†	-7.89±0.23

Groups	%Maximum response SNP	ED_{50} (log M) SNP
Intact Control	99.98±3.26	-6.89±0.09
OVX	94.00±1.25	-6.89±0.11
OVX+ Estrogen	94.29±1.36	-7.34±0.13
OVX+*P. mirifica*	97.92±3.23	-6.89±0.14

Data are expressed as mean±S.E.M.

* $P < 0.05$ compared to control group.

† $P < 0.05$ compared to OVX group.

2.3 Vascular structural changes.

The integrity of the endothelial cell lining was evaluated under scanning electron microscope. Representative scanning electron micrographs taken from aorta in this study are shown in Fig 4.7 . The control group had normal endothelial cell alignment in the direction of flow with no intercellular gaps. In contrast, vessel endothelium from cholesterol group demonstrates a loss of flow alignment and occasional intercellular gaps. Raised area inside the rectangular may indicate area of subendothelial foam cell formation (atheroma). Adherent of a large number of WBC are clustered on the surface of the endothelium. Whereas, endothelial cells morphology appears normal in OVX+Estrogen and OVX+*P.* mirifica groups.

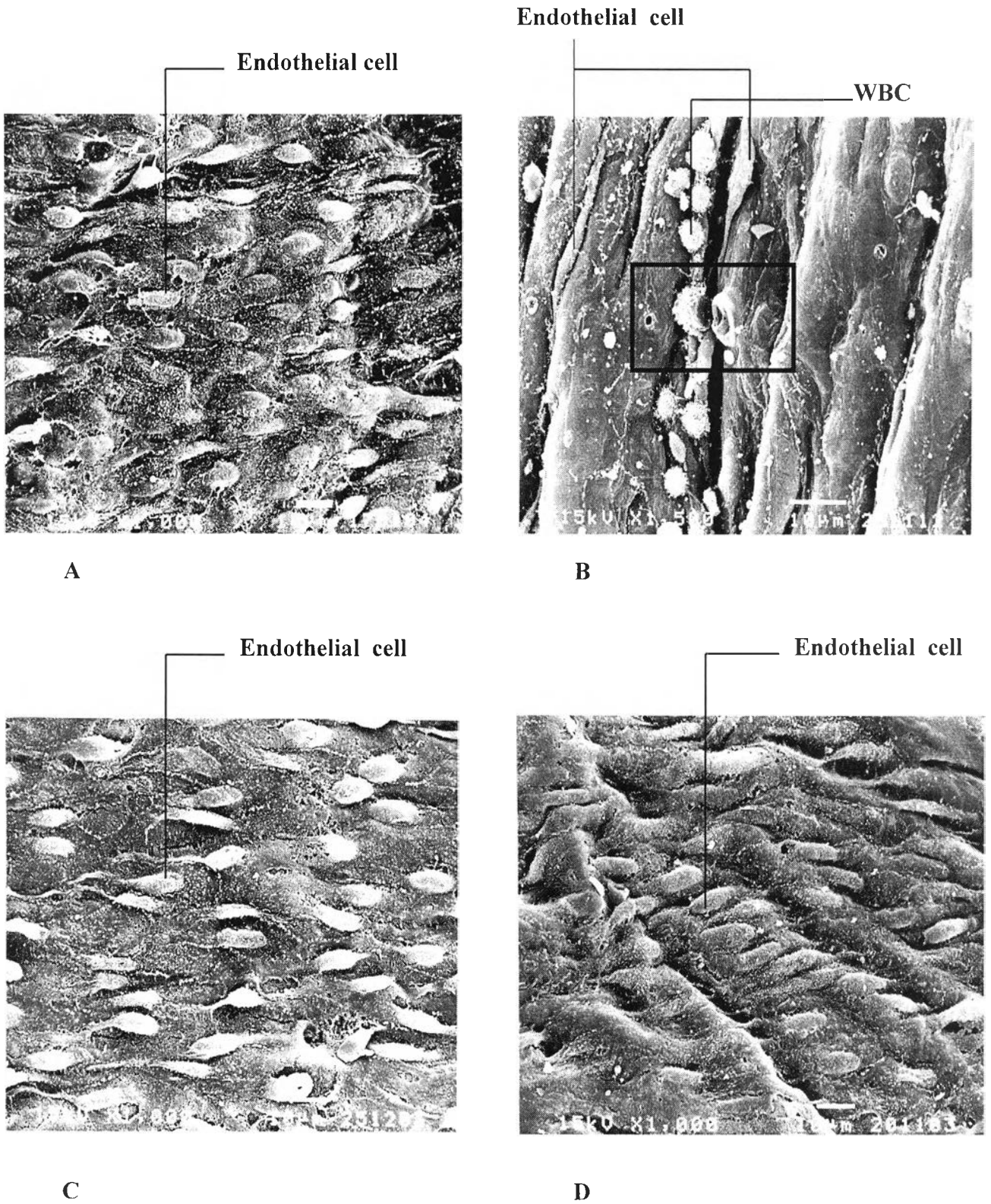

Fig 4.7 Scanning electron micrographs of aortic endothelium from Intact control group (A), OVX group (B), OVX+estrogen group (C) and OVX+*P. mirifica* group (D).

Bar 10 μm, magnification (A,C,D X 1000), (B X 1500).