CHAPTER III

RESULT

3.1 Rat's body weight

The effects of ginger diet and methyl parathion on rats body weight were shown in Table 1 to Table 6. The body weight of the animals in each group was increased at the end of experiment. The body weight gain was not statistically significant between the groups.

Several effects were observed in the rats exposed to methyl parathion. The first observed effects were usually fine body tremors, fasciculation, lacrimination, salivation, abdominal cramp low motor activity, legs paralysis and very aggressive behavior. The symptoms were recorded in standard working sheet of Hippocratic screening test. No death was found among rats tested.

Table 1 The body weight of male rats treated with 0.5, 1.0 and 1.5 mg/kg methyl parathion (values are expressed as mean±SD, n=6)

Dose	Time	Body weight before experiment (gram)	Body weight after experiment (gram)
Control propylene glycol	Week1	191.66 ± 8.75	220.00 ±12.64*
	Week4	195.83 ± 15.94	312.50 ±12.54*
Methyl parathion 0.5 mg/kg	Week1	191.66 ± 7.52	235.83 ±21.31*
	Week4	190.00 ± 7.07	305.83 ± 4.91*
Methyl parathion 1.0 mg/kg	Week1	195.83 ± 3.76	216.66 ± 6.05*
	Week4	189.16 ± 7.35	303.33 ± 5.16*
Methyl parathion 1.5 mg/kg	Week1	183.33 ± 7.52	210.83 ± 4.91*
	Week4	198.33± 5.16	296.66 ±19.66*

* Significantly different from the body weight before experiment ($P<0.05$)

Table 2 The body weight of female rats treated with 0.5, 1.0 and 1.5 mg/kg methyl parathion (values are expressed as mean±SD, n=6)

Dose	Time	Body weight before experiment (gram)	Body weight after experiment (gram)
Control propylene glycol	Week1	172.50 ± 1.48	206.66 ± 7.52*
	Week4	184.16 ± 7.35	235.83 ± 14.28*
Methyl parathion 0.5 mg/kg	Week1	185.00 ± 6.32	210.00 ± 5.47*
	Week4	175.83 ± 5.84	215.83 ± 6.64*
Methyl parathion 1.0 mg/kg	Week1	179.11 ± 7.35	198.33 ± 4.08*
	Week4	188.33 ± 5.16	220.83 ± 13.93*
Methyl parathion 1.5 mg/kg	Week1	195.00 ± 4.47	221.66 ± 8.75*
	Week4	181.66 ± 5.16	223.33 ± 11.69*

* Significantly different from the body weight before experiment ($P<0.05$)

Table 3 The body weight of male rats treated with 0.5, 1.0 and 1.5 %W/W ginger diet (values are expressed as mean±SD, n=6)

Dose	Time	Body weight before experiment (gram)	Body weight after experiment (gram)
Normal diet	Week1	193.33 ± 6.05	280.83 ± 17.72*
	Week4	191.25 ± 8.73	301.56 ± 14.32*
0.5%W/W ginger diet	Week1	194.16 ± 8.01	268.33 ± 13.29*
	Week4	192.55 ± 8.97	290.45 ± 20.36*
1.0%W/W ginger diet	Week1	195.83 ± 8.01	285.00 ± 10.48*
	Week4	195.77 ± 10.38	295.46 ± 9.66*
1.5%W/W ginger diet	Week1	189.16 ± 10.20	278.33 ± 13.29*
	Week4	190.25 ± 8.42	310.44 ± 10.28*

* Significantly different from the body weight before experiment ($P<0.05$)

Table 4 The body weight of female rats treated with 0.5, 1.0 and 1.5 %W/W ginger diet (values are expressed as mean±SD, n=6)

Dose	Time	Body weight before experiment (gram)	Body weight after experiment (gram)
Normal diet	Week1	190.00 ± 7.07	206.66 ± 8.16*
	Week4	188.72 ± 8.35	250.44 ± 8.75*
0.5%W/W ginger diet	Week1	190.83 ± 9.17	217.50 ± 7.58*
	Week4	187.40 ± 8.56	250.64 ± 8.79*
1.0%W/W ginger diet	Week1	185.83 ± 9.17	235.00 ± 12.24*
	Week4	195.66 ± 8.54	260.47 ± 9.54*
1.5%W/W ginger diet	Week1	186.66 ± 10.32	268.33 ± 27.14*
	Week4	185.66 ± 8.66	255.68 ± 12.14*

* Significantly different from the body weight before experiment ($P<0.05$)

Table 5 The body weight of male rats treated with 1.0 mg/kg methyl parathion and 1.5 %W/W ginger diet (values are expressed as mean±SD, n=6)

Dose	Time	Body weight before experiment (gram)	Body weight after experiment (gram)
Normal diet	Week4	191.66± 8.54	311.56 ± 10.39*
1.5%W/W ginger diet	Week4	182.55 ± 8.97	290.78 ± 20.32*
1.0 mg/kg methyl parathion + 1.5%W/W ginger diet	Week4	185.77 ± 10.38	290.46 ± 8.65*
1.0 mg/kg methyl parathion	Week4	185.66 ± 9.87	305.48 ± 10.28*

* Significantly different from the body weight before experiment ($P<0.05$)

Table 6 The body weight of female rats treated with 1.0 mg/kg methyl parathion and 1.5 %W/W ginger diet (values are expressed as mean±SD, n=6)

Dose	Time	Body weight before experiment (gram)	Body weight after experiment (gram)
Normal diet	Week4	181.72± 8.37	260.44 ± 8.74*
1.5%W/W ginger diet	Week4	187.36 ± 8.54	260.64 ± 8.00*
1.0 mg/kg methyl parathion + 1.5%W/W ginger diet	Week4	195.56 ± 10.54	270.47 ± 9.50*
1.0 mg/kg methyl parathion	Week4	182.66 ± 9.60	280.38 ± 12.42*

* Significantly different from the body weight before experiment ($P<0.05$)

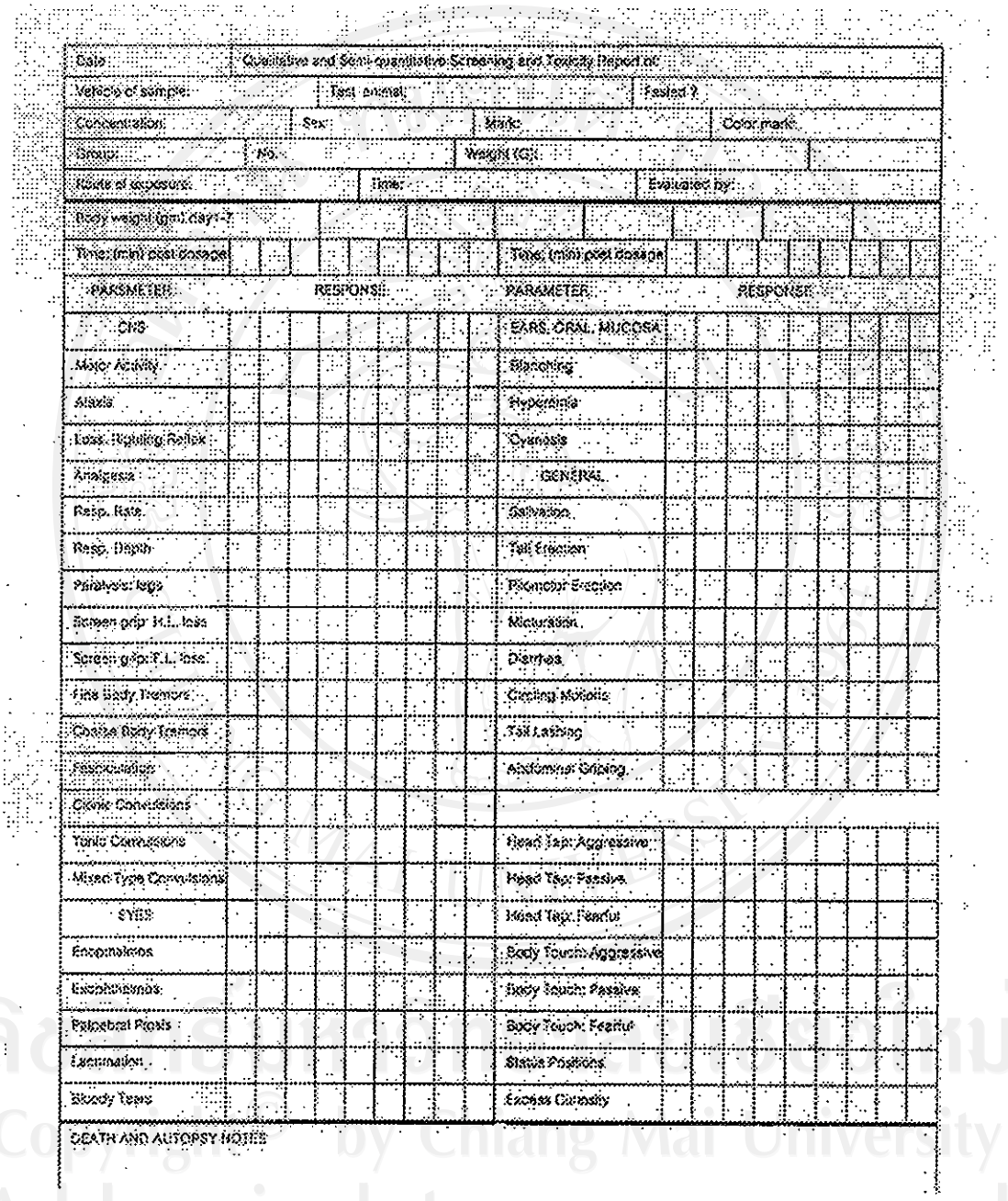

Figure 20 Standard working sheet of Hippocratic screening test

3.2 Selection of the optimal dose of methyl parathion

1.0 mg/kg of methyl parathion was selected as the optimal dose for treating the animals for 4 weeks. The animals showed little signs of acute toxicity (e.g. tremors, chewing and lacrimination) and the AChE level was significantly decreased. Methyl parathion treatment enhanced lipid peroxidation in rats fed with normal diet when compared to controls. The blood glutathione content in methyl parathion treatment was significantly reduced. Methyl parathion was significantly enhanced both the GST and GR activity in rats fed with normal diet. The results are shown in Table 7-8 and Figure 21-30.

Table 7 The amount of TBARS, AChE, GSH, GST and GR in male rats exposed to 0.5, 1.0 and 1.5 mg/kg methyl parathion (values are expressed as mean±SD, n=6)

Dose	Time	TBARS (mM)	AChE (U/L)	GSH (mg/dl of whole blood)	GST (Units)	GR (units/ml)
Control propylene glycol	Week1	20.81 ± 1.01	2000.00 ± 309.83	16.35 ± 2.00	0.43 ± 0.04	3.34 ± 0.33
	Week4	27.90 ± 5.78	2333.22 ± 273.25	21.56 ±2.17	0.37 ± 0.06	4.04 ± 0.58
Methyl parathion 0.5 mg/kg	Week1	17.95 ± 3.22	2166.66 ±294.39	17.21 ±2.43	0.24 ± 0.03*	3.18 ± 0.69
	Week4	22.97 ± 3.68*	1566.66 ±150.54*	20.27 ± 1.71	0.48 ± 0.06	4.80 ± 0.88
Methyl parathion 1.0 mg/kg	Week1	17.43± 1.82	2200.00 ± 644.98	17.27 ± 2.57	0.25 ± 0.01*	2.76 ± 0.87
	Week4	66.06 ± 5.79*	1366.66 ±150.55*	7.01 ± 2.55*	0.46 ± 0.08	4.14 ± 0.72
Methyl parathion 1.5 mg/kg	Week1	17.69 ± 1.93	1900.00 ±109.54	18.04 ± 1.68	0.36 ± 0.01	4.60 ± 0.48*
	Week4	48.58 ± 3.16*	1133.22 ±103.28*	6.43 ± 2.70*	0.49 ± 0.07*	5.09 ± 1.19

* Significantly different from control (P<0.05)

Table 8 The amount of TBARS, AChE, GSH, GST and GR in female rats exposed to 0.5, 1.0 and 1.5 mg/kg methyl parathion (values are expressed as mean±SD, n=6)

Dose	Time	TBARS (mM)	AChE (U/L)	GSH (mg/dl of whole blood)	GST (Units)	GR (units/ml)
Control propylene glycol	Week1	19.61 ± 1.34	2600.00 ± 419.52	22.03 ± 10.23	0.22 ± 0.03	3.74 ± 0.52
	Week4	33.94 ± 6.27	2833.20 ± 445.72	20.23 ± 0.96	0.31 ± 0.04	4.20 ± 0.49
Methyl parathion 0.5 mg/kg	Week1	19.87 ± 2.51	1733.22 ±242.21*	16.21 ± 1.52	0.22 ± 0.02	3.22 ± 0.57
	Week4	62.07 ± 6.53*	1933.20 ± 350.32*	7.84 ± 0.81*	0.48 ± 0.05*	4.15 ± 0.22
Methyl parathion 1.0 mg/kg	Week1	18.37 ± 2.01	1933.20 ± 163.33*	17.43± 0.64	0.34 ± 0.11	3.90 ± 0.81
	Week4	56.15 ± 8.10*	1533.22 ±103.28*	6.81 ± 1.83*	0.46 ± 0.08*	4.44 ± 0.61
Methyl parathion 1.5 mg/kg	Week1	21.51 ± 0.87	1900.66 ±105.55*	17.69 ± 1.53	0.36 ± 0.14	4.80 ± 0.82
	Week4	52.15 ± 1.56*	1500.00 ±209.76*	3.57 ± 0.49*	0.49 ± 0.07*	4.97 ± 0.23*

* Significantly different from control ($P<0.05$)

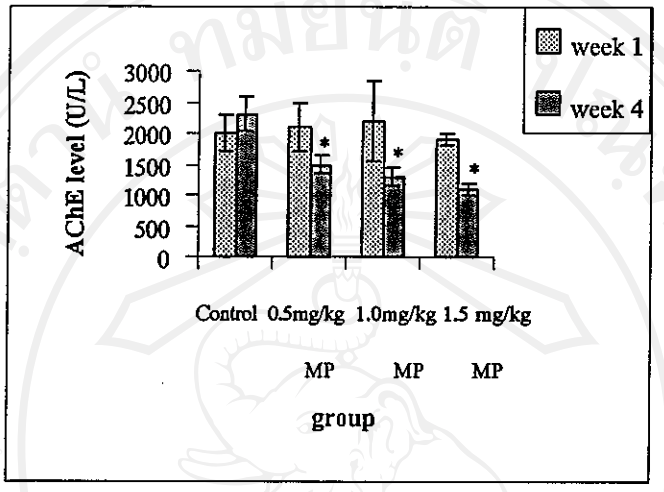

Figure 21 Red blood cell AChE levels in male rats exposed to 0.5, 1.0 and 1.5 mg/kg methyl parathion (values are expressed as mean±SD, n=6)

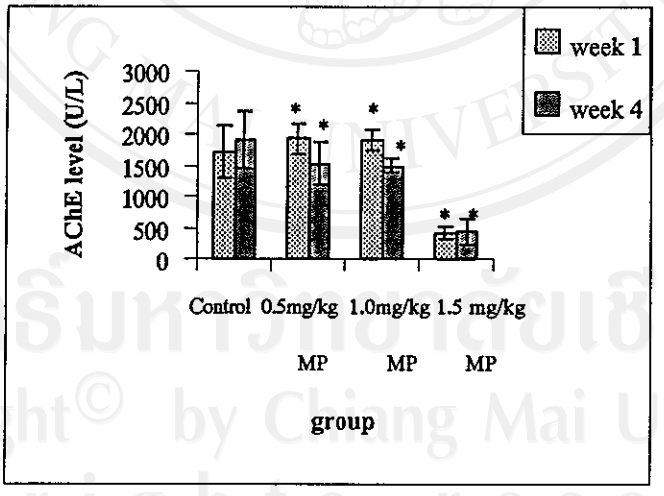

Figure 22 Red blood cell AChE levels in female rats exposed to 0.5, 1.0 and 1.5 mg/kg methyl parathion (values are expressed as mean±SD, n=6)

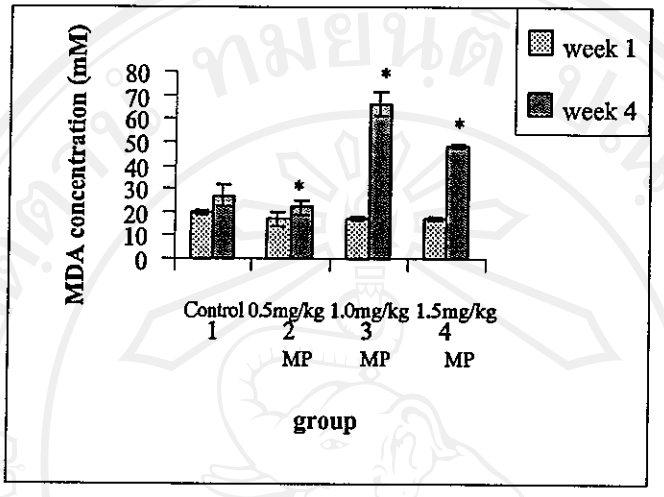

Figure 23 Serum TBARS concentration (MDA) in male rats exposed to 0.5, 1.0 and 1.5 mg/kg methyl parathion (values are expressed as mean±SD, n=6)

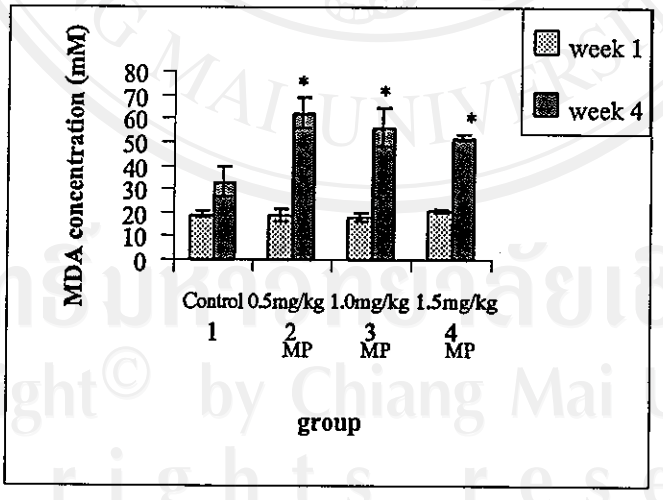

Figure 24 Serum TBARS concentration (MDA) in female rats exposed to 0.5, 1.0 and 1.5 mg/kg methyl parathion (values are expressed as mean±SD, n=6)

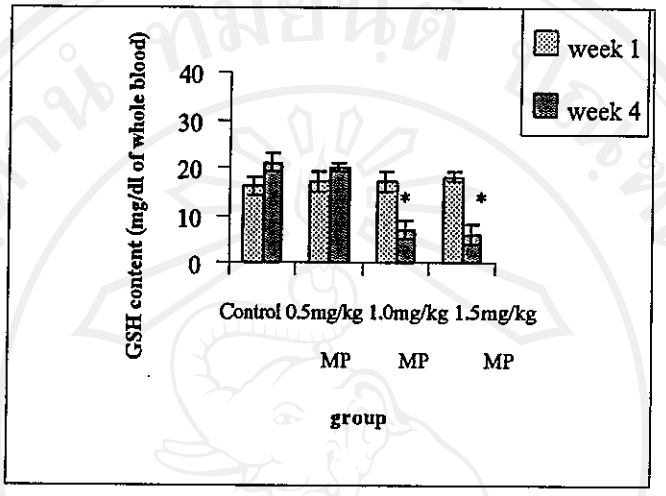

Figure 25 Red blood cell GSH content in male rats exposed to 0.5, 1.0 and 1.5 mg/kg methyl parathion (values are expressed as mean±SD, n=6)

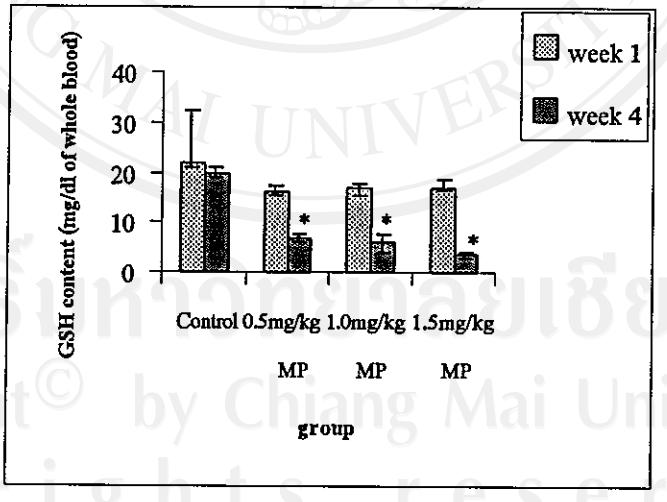

Figure 26 Red blood cell GSH content in female rats exposed to 0.5, 1.0 and 1.5 mg/kg methyl parathion (values are expressed as mean±SD, n=6)

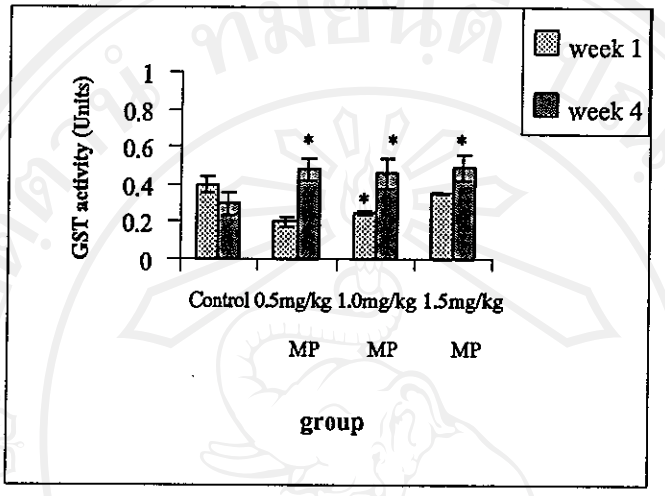

Figure 27 Liver GST activity in male rats exposed to 0.5, 1.0 and 1.5 mg/kg methyl parathion (values are expressed as mean±SD, n=6)

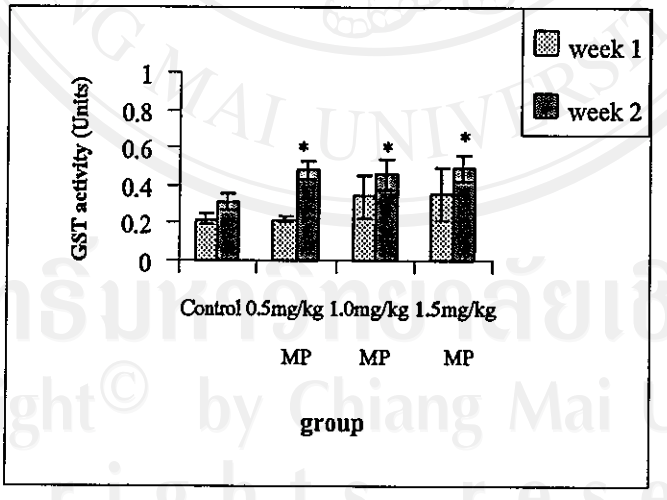

Figure 28 Liver GST activity in female rats exposed to 0.5, 1.0 and 1.5 mg/kg methyl parathion (values are expressed as mean±SD, n=6)

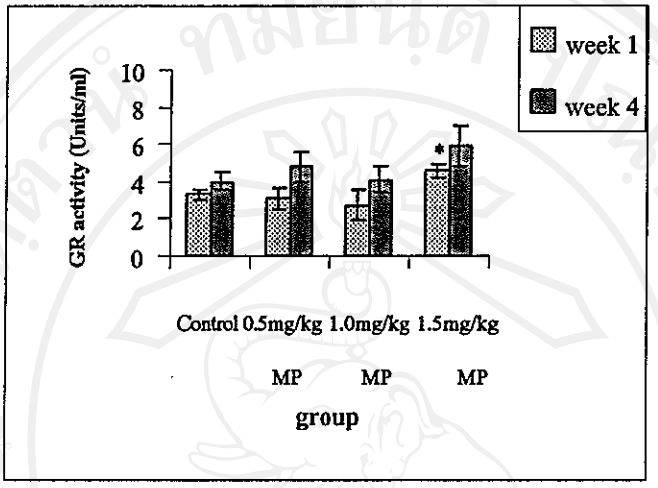

Figure 29 Liver GR activity in male rats exposed to 0.5, 1.0 and 1.5 mg/kg methyl parathion (values are expressed as mean±SD, n=6)

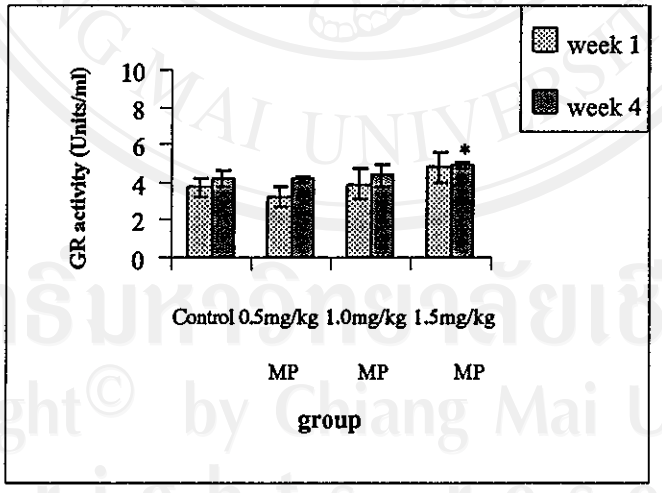

Figure 30 Liver GR activity in female rats exposed to 0.5, 1.0 and 1.5 mg/kg methyl parathion (values are expressed as mean±SD, n=6)

3.3 Selection of the the optimal dose of diet ginger

1.5%W/W ginger diet was selected as the optimal dose for treating the animals for 4 weeks. The AChE level in male rats treated with 1.5%W/W ginger diet was not significant from that of controls, but little increased AChE level was found in female rats. The GSH content in animals treated with ginger diet was significantly increased. Ginger diet enhanced GR activity, but not GST activity when compared to controls. The results are shown in Table 9-10 and Figure 31-40.

Table 9 The amount of TBARS, AChE, GSH, GST and GR in male rats exposed to 0.5, 1.0 and 1.5%W/W ginger diet (values are expressed as mean±SD, n=6)

Dose	Time	TBARS (mM)	AChE (U/L)	GSH (mg/dl of whole blood)	GST (Units)	GR (units/ml)
Normal diet	Week1	14.04 ± 4.42	2266.66 ± 628.22	25.12± 1.62	0.37± 0.05	3.63 ± 0.65
	Week4	34.06 ± 3.32	2266.66 ± 163.33	30.34 ±2.38	0.42 ± 0.04	3.95 ± 0.43
0.5% ginger diet	Week1	16.05 ± 1.80	2200.00 ± 309.83	27.81 ±2.2085	0.19 ± 0.05*	3.31 ± 0.30
	Week4	31.08 ± 2.11	2066.66 ± 546.50	38.73 ±2.64*	0.39 ± 0.06	3.99 ±0.68
1.0% ginger diet	Week1	10.08 ± 0.48*	2300.00 ± 563.38	30.08 ± 2.80*	0.27 ± 0.09	2.96 ± 0.35
	Week4	34.24 ± 2.80	2266.66 ± 413.11	35.35 ± 0.80*	0.32 ± 0.08	3.92 ± 0.48
1.5% ginger diet	Week1	10.10 ± 0.86*	2000.00 ± 219.09	28.29 ±1.69	0.26 ± 0.10	4.73 ± 0.96*
	Week4	27.02 ± 2.64*	2500.00 ± 562.13	37.41 ±1.69*	0.47 ± 0.09	5.27 ± 1.05*

* Significantly different from control ($P<0.05$)

Table 10 The amount of TBARS, AChE, GSH, GST and GR in female rats exposed to 0.5, 1.0 and 1.5%W/W ginger diet (values are expressed as mean±SD, n=6)

Dose	Time	TBARS (mM)	AChE (U/L)	GSH (mg/dl of whole blood)	GST (Units)	GR (units/ml)
Normal diet	Week1	16.33 ±4.21	2400.00 ± 357.77	22.13 ±3.34	0.23 ± 0.02	3.82 ±0.59
	Week4	27.59 ±3.34	2266.66 ± 326.59	30.19 ± 3.42	0.39 ± 0.03	4.70 ± 0.58
0.5% ginger diet	Week1	10.76 ±2.04*	1933.22 ± 413.11	25.91 ± 2.49	0.28 ± 0.07	3.04 ± 0.11
	Week4	31.11 ±2.17	2266.66 ± 546.50	40.93 ± 2.35*	0.30 ± 0.10	5.72 ± 0.46*
1.0% ginger diet	Week1	7.13 ±1.613*	2100.00 ± 209.76	24.82 ± 2.68	0.28 ± 0.07	3.40 ± 0.77
	Week4	26.08 ±9.53	2566.66 ± 571.54	39.44 ± 2.82*	0.36 ± 0.06	4.00 ± 0.41
1.5% ginger diet	Week1	13.51± 1.27	2066.66 ± 372.38	30.24 ± 6.58*	0.27 ± 0.09	5.43 ± 0.57*
	Week4	24.70 ±2.94	3133.22 ± 372.38	39.01 ± 1.80*	0.36 ± 0.07	5.63 ± 0.62*

* Significantly different from control ($P<0.05$)

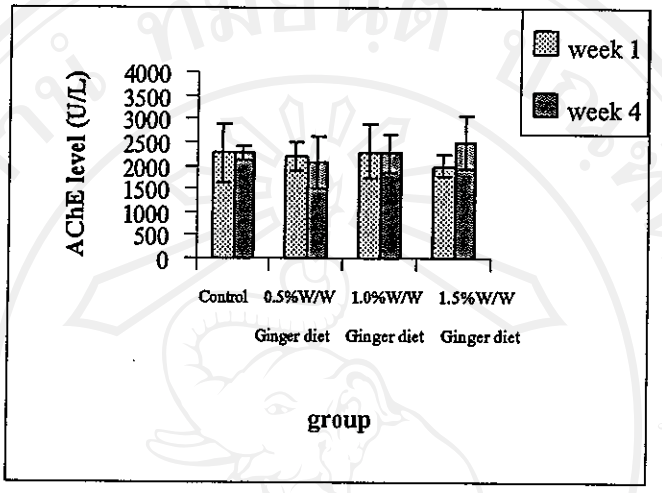

Figure 31 Red blood cell AChE levels in male rats exposed to 0.5, 1.0 and 1.5%W/W ginger diet (values are expressed as mean±SD, n=6)

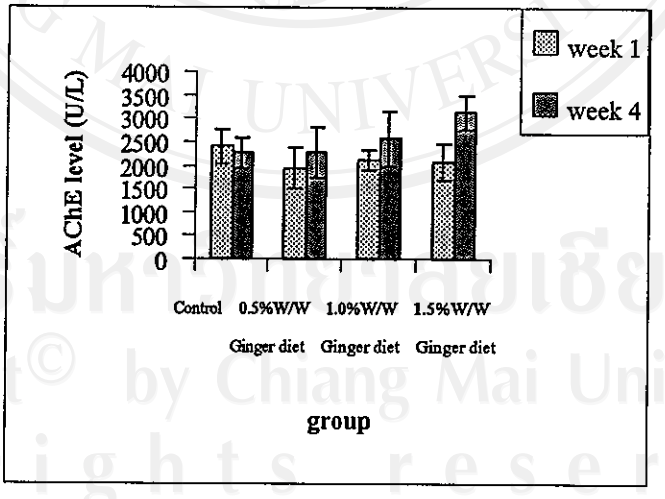

Figure 32 Red blood cell AChE levels in female rats exposed to 0.5, 1.0 and 1.5%W/W ginger diet (values are expressed as mean±SD, n=6)

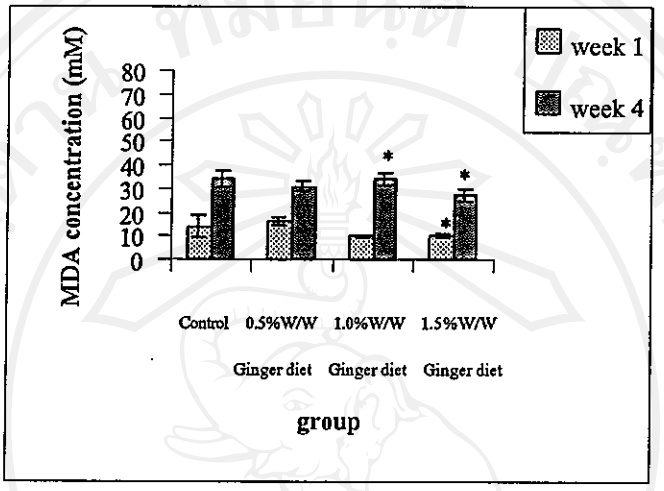

Figure 33 Serum TBARS concentration (MDA) in male rats exposed to 0.5, 1.0 and 1.5%W/W ginger diet (values are expressed as mean±SD, n=6)

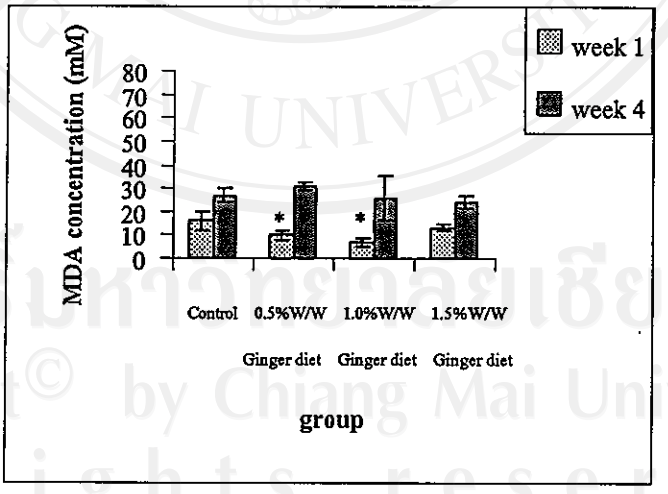

Figure 34 Serum TBARS concentration (MDA) in female rats exposed to 0.5, 1.0 and 1.5%W/W ginger diet (values are expressed as mean±SD, n=6)

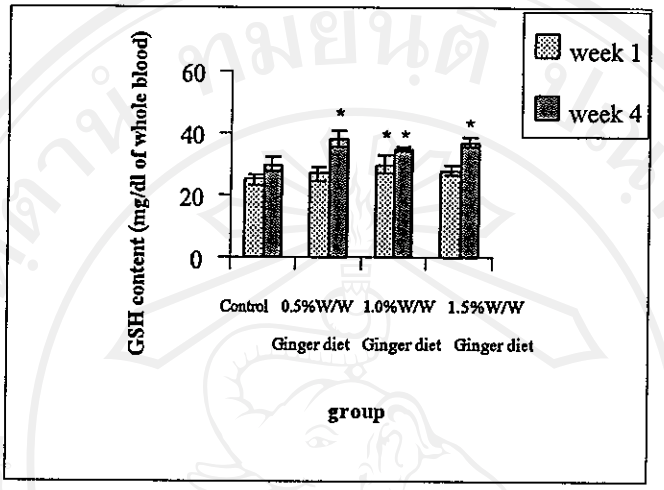

Figure 35 Red blood cell GSH content in male rats exposed to 0.5, 1.0 and 1.5%W/W ginger diet (values are expressed as mean±SD, n=6)

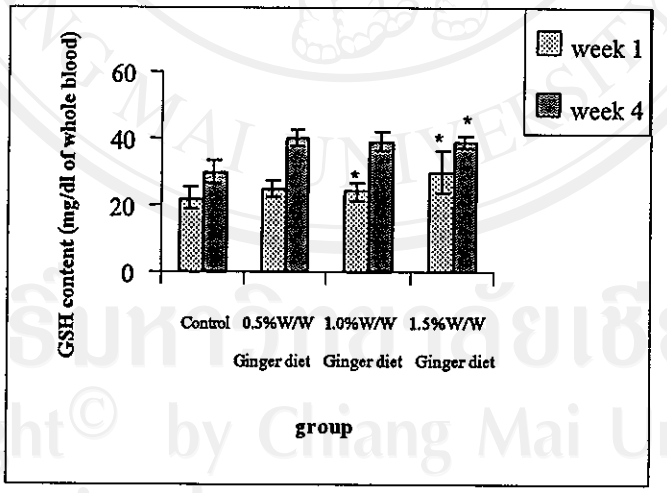

Figure 36 Red blood cell GSH content in female rats exposed to 0.5, 1.0 and 1.5%W/W ginger diet (values are expressed as mean±SD, n=6)

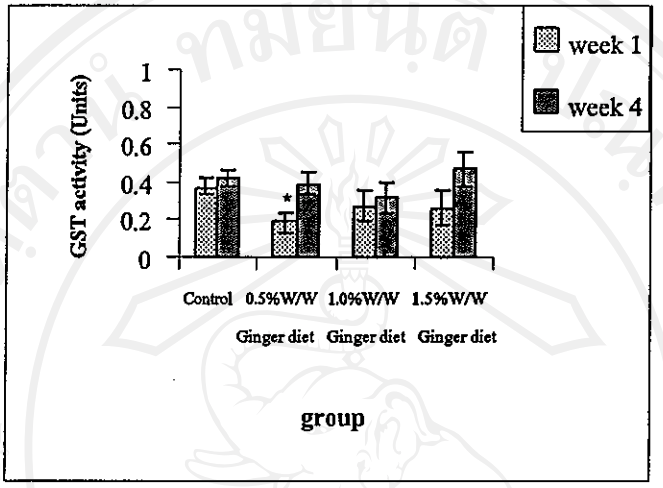

Figure 37 Liver GST activity in male rats exposed to 0.5, 1.0 and 1.5%W/W ginger diet (values are expressed as mean±SD, n=6)

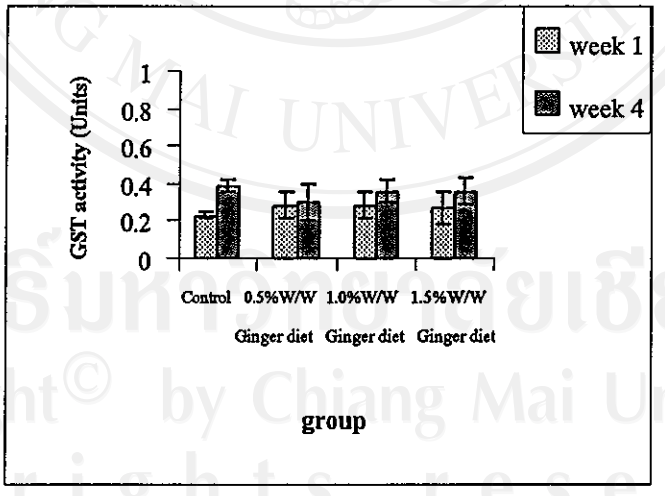

Figure 38 Liver GST activity in female rats exposed to 0.5, 1.0 and 1.5%W/W ginger diet (values are expressed as mean±SD, n=6)

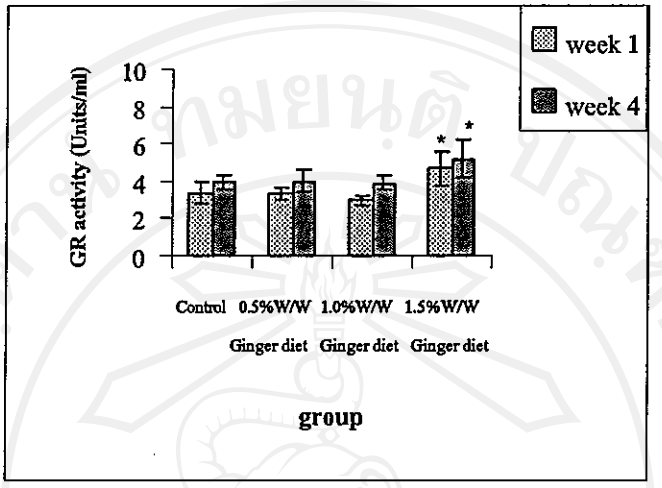

Figure 39 Liver GR activity in male rats exposed to 0.5, 1.0 and 1.5%W/W ginger diet (values are expressed as mean±SD, n=6)

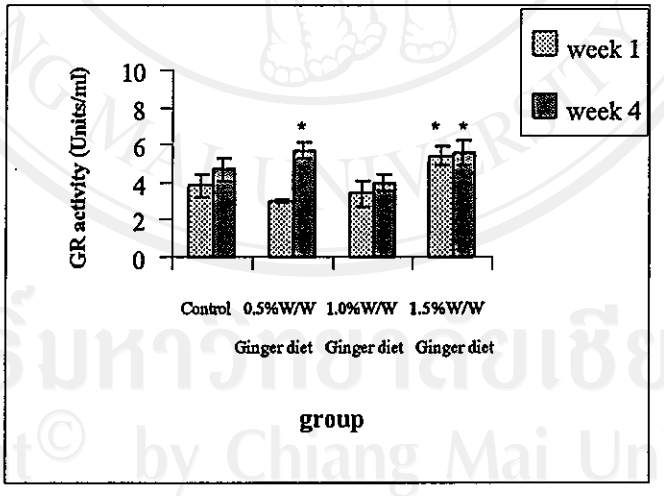

Figure 40 Liver GR activity in female rats exposed to 0.5, 1.0 and 1.5%W/W ginger diet (values are expressed as mean±SD, n=6)

3.4 Effects of methyl parathion and ginger diet

The animals treated with 1.0 mg/kg methyl parathion showed little sign of acute toxicity and enhanced lipid peroxidation when compared to those of animals treated with both of 1.0 mg/kg methyl parathion and 1.5%W/W ginger diet. The AChE level was declined in animals treated with 1.0 mg/kg methyl parathion when compared to that of the controls. The content of GSH in animals treated with both 1.0 mg/kg methyl parathion and 1.5%W/W ginger diet was increased significantly when compared to that of animals treated with 1.0 mg/kg methyl parathion. The GST and GR activity were increased significantly when compared to those of controls. The results are shown in Table 11-12 and Figure 41-45.

Table 11 The amount of TBARS, AChE, GSH, GST and GR in male rats treated with 1.0 mg/kg methyl parathion and 1.5 %W/W ginger diet for 4 weeks (values are expressed as mean±SD, n=6)

Group	TBARS (mM)	AChE (U/L)	GSH (mg/dl of whole blood)	GST (Units)	GR (Units/ml)
Control	19.21 ± 1.29	2700.00 ± 209.76	18.98 ± 1.22	0.30 ± 0.02	2.23 ± 0.35
1.5%W/W Ginger diet	24.00 ± 3.48	2333.22 ± 206.55	30.79 ± 1.57*	0.34 ± 0.02	2.49 ± 0.35
1.0 mg/kg methyl parathion	48.01 ± 2.98*	1500.00 ± 167.33*	5.74 ± 0.39*	0.50 ± 0.05*	2.56 ± 0.39
1.5%W/W Ginger diet +1.0 mg/kg methyl parathion	28.68 ± 5.26*	1466.66 ± 81.64*	34.83 ± 2.85*	0.48 ± 0.09*	3.42 ± 0.52*

* Significantly different from control ($P<0.05$)

Table 12 The amount of TBARS, AChE, GSH, GST and GR in female rats treated with 1.0 mg/kg methyl parathion and 1.5 %W/W ginger diet for 4 weeks (values are expressed as mean±SD, n=6)

Group	TBARS (mM)	AChE (U/L)	GSH (mg/dl of whole blood)	GST (Units)	GR (Units/ml)
Control	19.16 ± 1.49	3133.22 ± 273.25	19.66 ± 1.88	0.30 ± 0.04	2.46 ± 0.52
1.5%W/W Ginger diet	25.80 ± 2.92	3133.22 ± 163.29	31.69 ± 3.76*	0.34 ± 0.03	2.87 ± 0.20
1.0 mg/kg methyl parathion	48.23 ± 3.22*	1466.66 ± 206.55*	6.6463 ± 0.88*	0.52 ± 0.06*	3.12 ± 0.36
1.5%W/W Ginger diet +1.0 mg/kg methyl parathion	28.59 ± 2.94*	1500.00 ± 200.00*	36.90 ± 1.80*	0.47 ± 0.04*	3.35 ± 0.41*

* Significantly different from control ($P<0.05$)

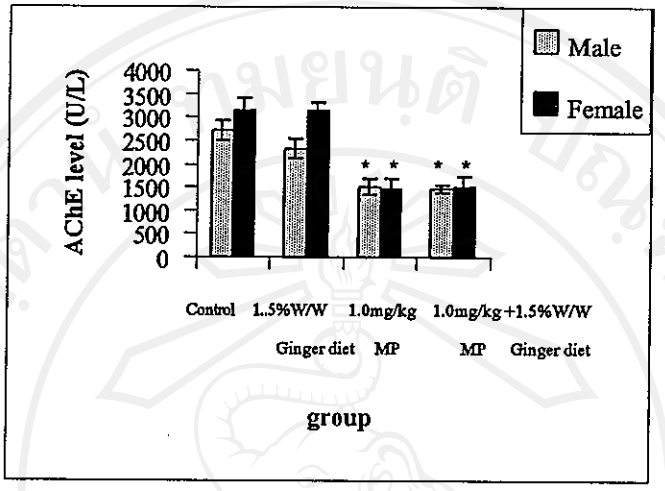

Figure 41 Red blood cell AChE level in male and female rats treated with 1.0 mg/kg methyl parathion and 1.5 %W/W ginger diet for 4 weeks (values are expressed as mean±SD, n=6)

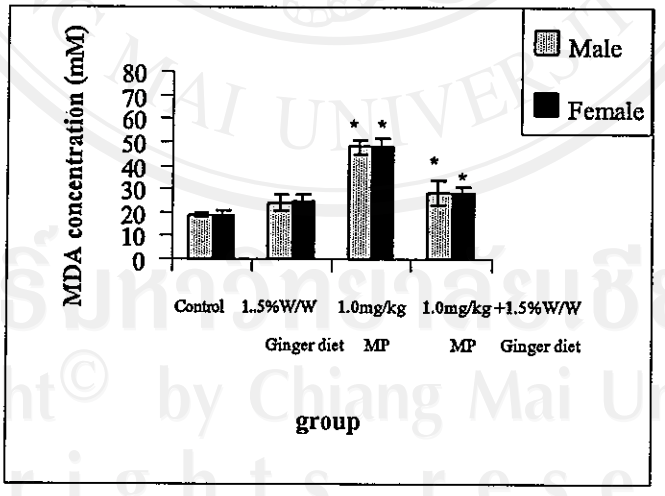

Figure 42 Serum TBARS concentration (MDA) in male and female rats treated with 1.0 mg/kg methyl parathion and 1.5 %W/W ginger diet for 4 weeks (values are expressed as mean±SD, n=6)

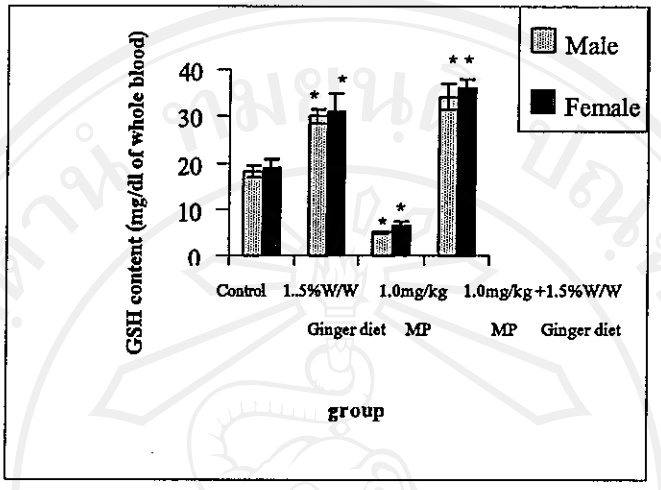

Figure 43 Red blood cell GSH content in male and rats treated with 1.0 mg/kg methyl parathion and 1.5 %W/W ginger diet for 4 weeks (values are expressed as mean±SD, n=6)

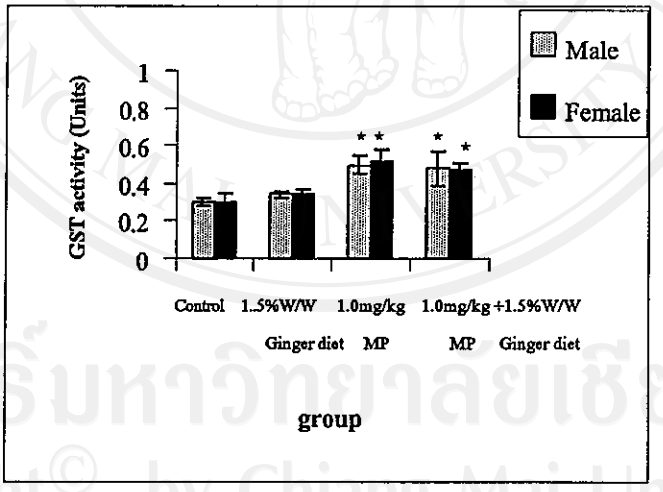

Figure 44 Liver GST activity in male and female rats treated with 1.0 mg/kg methyl parathion and 1.5 %W/W ginger diet for 4 weeks (values are expressed as mean±SD, n=6)

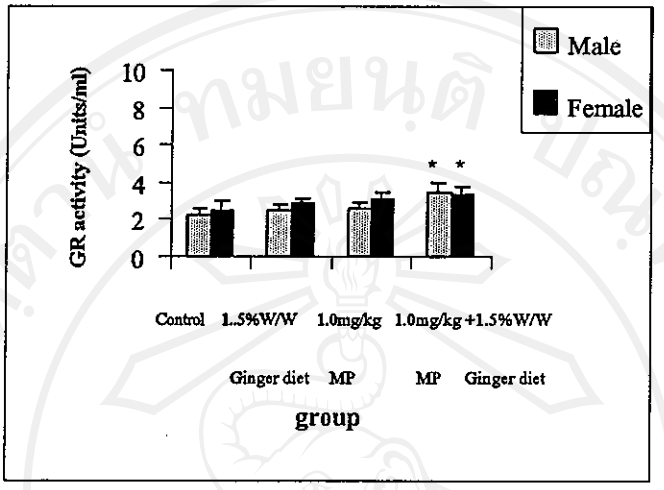

Figure 45 Liver GR activity in male and rats treated with 1.0 mg/kg methyl parathion and 1.5 %W/W ginger diet for 4 weeks (values are expressed as mean±SD, n=6)

3.5 Acetylcholinesterase activity in muscle

The number of motor endplate positive acetylcholinesterase activity was unchanged in animals treated with 1.5%W/W ginger diet and control diet but was decreased significantly in both group of animals treated with 1.0 mg/kg methyl parathion and 1.5%W/W ginger diet plus with 1.0 mg/kg MP when compared to that of control. The results are shown in Table 13.

Controls showed unchanged staining intensity of motor endplate and sharply defined granular reddish brown precipitate. The morphology of motor endplate of animals treated with 1.5%W/W ginger diet was unchanged when compared to that of controls. The results are shown in Figure 47.

The morphology of motor endplate of animals treated with 1.0 mg/kg methyl parathion and 1.5%W/W ginger diet plus with 1.0 mg/kg MP showed distortion and edema of motor endplate. The result are shown in Figure 48. Ginger diet supplement could not improved AChE activity of motor endplate in muscle.

Table 13 The number of motor endplate positive AChE activity in muscle of male and female rats treated with 1.0 mg/kg methyl parathion and 1.5 %W/W ginger diet for 4 weeks (values are expressed as mean±SD, n=6)

Group	Sex	No. of motor endplate positive AChE activity	Sex	No. of motor endplate positive AChE activity
Control	Male	140.50 ± 7.39	Female	143.66 ± 4.32
1.5%W/W Ginger diet	Male	141.33 ± 4.41	Female	147.50 ± 4.80
1.0 mg/kg methyl parathion	Male	71.83 ± 7.46*	Female	73.33 ± 7.63*
1.5%W/W Ginger diet +1.0 mg/kg methyl parathion	Male	77.66 ± 6.47*	Female	81.50 ± 4.50*

* Significantly different from control ($P<0.05$)

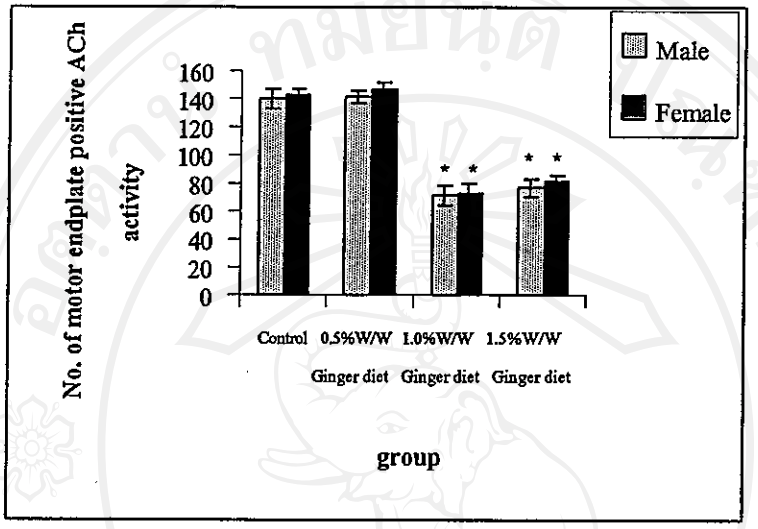

Figure 46 The number of motor endplate positive AChE activity in muscles of male and female rats treated with 1.0 mg/kg methyl parathion and 1.5 %W/W ginger diet for 4 weeks (values are expressed as mean±SD, n=6)

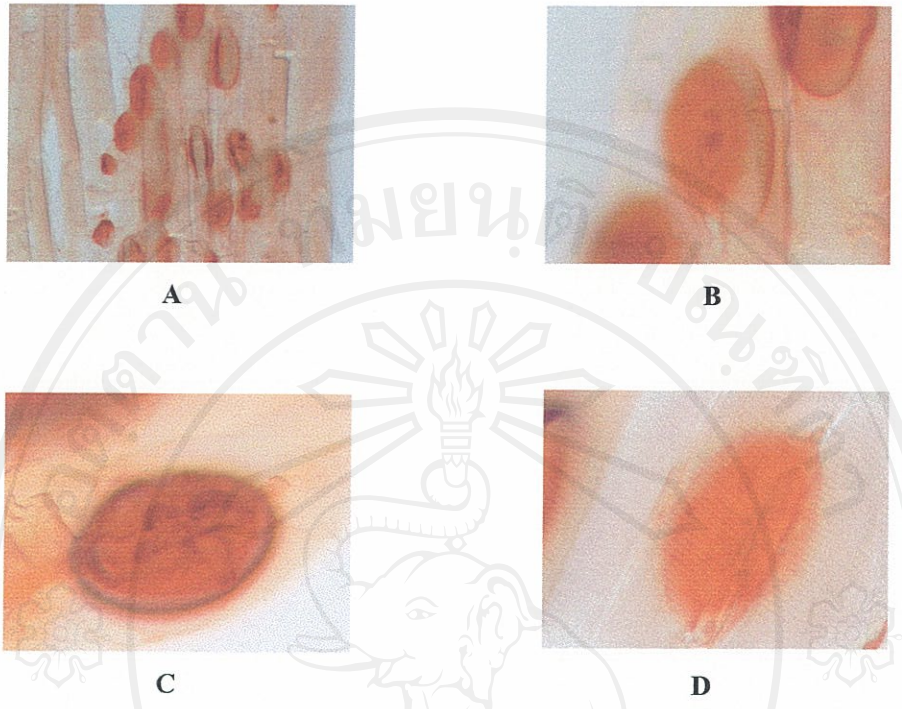

Figure 47 Normal motor endplate showing positive AChE activity in muscle of rat treated with normal diet and 1.5%W/W ginger diet. **(A):** Normal motor endplate with positive AChE activity in muscle of control rat treated with normal diet for 4 weeks. The positive AChE activity is bright sharply defined granular reddish brown precipitate. (x10), **(B):** (x40). Normally-appeared motor endplate positive AChE activity in muscle of control rat treated with 1.5%W/W ginger diet for 4 weeks. The endplate is intact with sharply defined granular precipitate **(C):** (X40) and shows irregular and blurred along the endplate border **(D):** (X40)

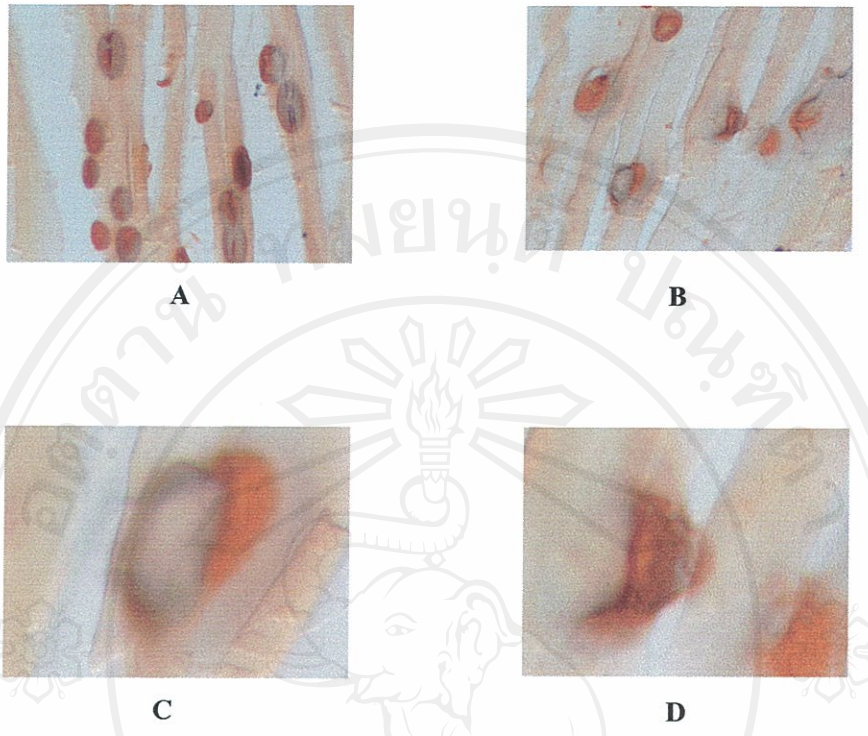

Figure 48 Low AChE activity of motor endplate in muscle of rat treated with 1.0 mg/kg methyl parathion and 1.5%W/W ginger diet plus with 1.0 mg/kg MP. The motor endplates are distorted and edematous. No granular reddish brown precipitate but thick linear staining along the borders of the endplate. **(A):** Low AChE activity of motor endplate in muscle of rat treated with 1.0 mg/kg methyl parathion plus 1.5%W/W ginger diet for 4 weeks. The number of motor endplate is not changed. Some motor endplates display normal staining granular pattern but the other disclose linear precipitates at the midportion of the endplates. (x10), Low AChE activity of motor endplate in muscle of rat treated with 1.0 mg/kg methyl parathion for 4 weeks. The distorted staining pattern with blurred border of the endplate **(B):** (x10), **(C):** (x40), **(D):** The motor endplate is distorted with thick linear staining pattern. No granular precipitate. (x40)

3.6 ABTS radical cation decolorization assay

Percent of reduction ABTS of ginger powder (20 mg/ml) and 1.5%W/W ginger diet as compared to that of Trolox, was found that, 80% of reduction ABTS equals to 0.24 mM of Trolox, 4.41 mg/ml of 1.5%W/W ginger diet and 7.7×10^{-4} mg/ml of ginger powder. Then, 1 gram of powder equals to 54.42 mM of Trolox.

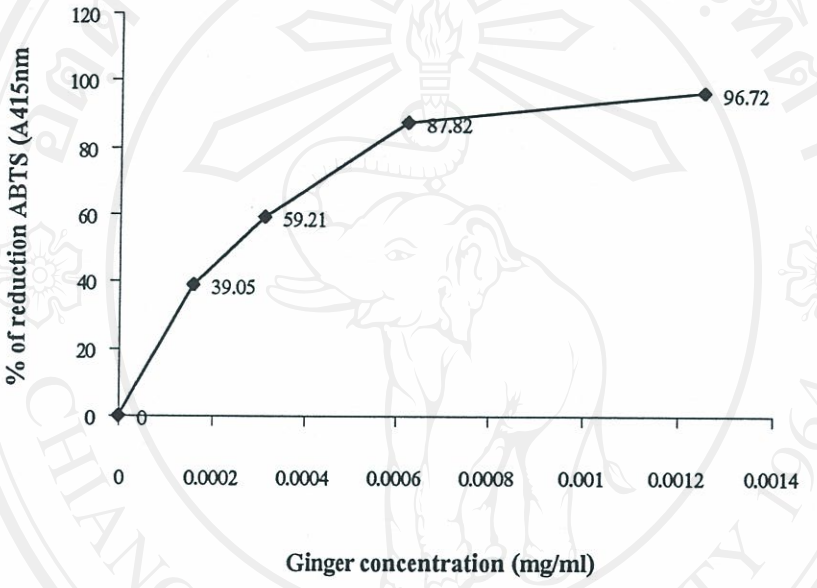

Figure 49 Percent reduction of ABTS by powder ginger

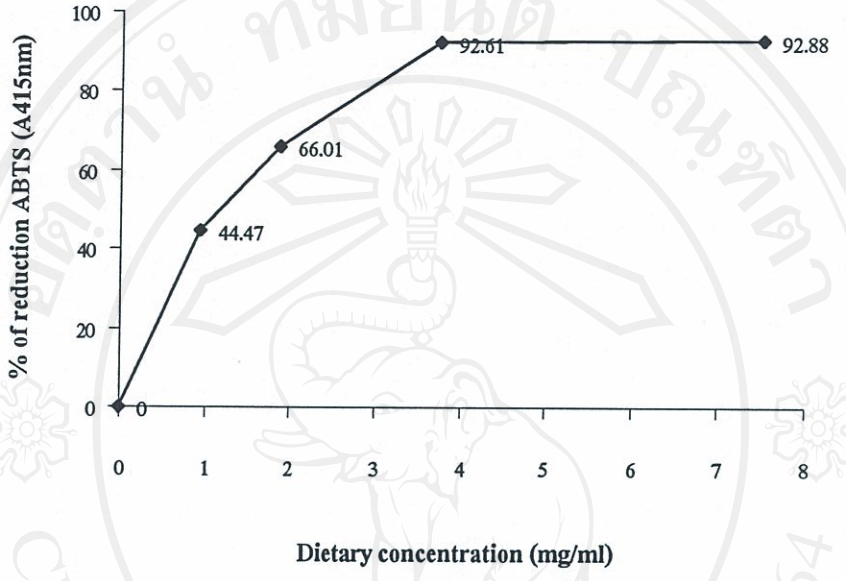

Figure 50 Percent reduction of ABTS by 1.5%W/W ginger diet