CHAPTER III

RESULTS

3.1 Carbon monoxide concentration in ambient air

Carbon monoxide concentrations at five sites in Chiang Mai; the Rin Kham, Khuang Sing and Juvenile Court intersections, Nawarat bridge and Warorod market was shown in **Appendix C.** The average concentration (mean ± SD) of carbon monoxide at all 5 heavy traffic areas in Chiang Mai was 3.71 ± 0.73 ppm **(Table 6).** Experimental dose used in this study was calculated using the following formula

$$\text{Experimental dose} = A \times B \times C$$

Where, A is the average concentration of carbon monoxide

B is uncertainty factor of safety factor between human and animal (10)

C is intraindividual difference factor (10)

So,

$$\text{Experimental dose} = 2.98 \times 10 \times 10 \text{ ppm}$$
$$= 298 \text{ ppm}$$

and

$$= 4.44 \times 10 \times 10 \text{ ppm}$$
$$= 444 \text{ ppm}$$

The experimental dose was calculated to be in between 298 and 444 ppm. Carbon monoxide generator was adjusted to produce carbon monoxide in between 298 and 444 ppm. The carbon monoxide concentration in animal chamber was detected while doing exposure experiment.

Table 6 The average of carbon monoxide concentration were measured at the 5 sites.

Sites	Mean ± SD (ppm)
Rin Kham	3.38 ± 0.82
Khung Sing	3.31 ± 0.77
Juvenile Court	3.74 ± 0.28
Nawarat bridge	4.43 ± 0.50
Warorod market	3.67 ± 0.60
Average / 5 sites	3.71 ± 0.73

3.2 Subchronic carbon monoxide exposure

After 3 months exposure to subchronic carbon monoxide low concentration rats were not died and appeared to be the same behavior as the control rats, except only sleepy sign that was noticed.

3.3 Pathology

All rat 's internal organs were examined by pathologists. The organs of interest were brain, heart, lungs, liver and kidneys, which would reflect tissue injury due to inhalation of carbon monoxide and partly absorbed through the circulation. However, there was no any abnormality detected grossly. The macroscopic appearances of these organs were unremarkable with smooth surfaces and homogeneous on their cut surfaces. Their architectures were within normal limits. Neither hemorrhage nor necrosis was observed. The colors of these organs were homogeneous and looked normal. No any significant alterations were noted between the controlled and treated animals.

Histologically, no any permanent cell injury was detected. The tissue architectures and their parenchyma were intact. No significant pathologic features seen. No any differences between the groups of the animals were observed.

The brains were unremarkable with intact cellular layers in the cerebral cortices. The basal nuclei were intact and the granular cell layers including Purkinje's cells in the cerebella hemisphere were well preserved. The arachnoid membrane was thin. Subarachnoid congestion was mild and unremarkable.

The lungs were intact with smooth pleural surface and patent bronchioles. The pulmonary lobules were well recognized without any cellular infiltration but mild congestion. The epithelial linings of the bronchioles were intact. Peribronchilar infiltration of lymphoid cells were occasionally observed in both groups and both sexes without distortion of alveolar spaces. The septae were thin and lined by flattened epithelial cells. Neither pulmonary infarct nor edema was observed.

The hearts were composed of intact myocardial fibers without either cardiac hypertrophy or infarcts.

The livers were intact both lobular architectures and the liver cell cords. Mild congestion was note. No definitive cellular injury was observed.

The renal glomeruli and tubes were well preserved with minimal interstitial congestion. Neither cellular casts nor cellular degeneration was noted. The renal capsule was thin and smooth.

3.4 Body weight of the carbon monoxide exposure rats

Body weight of the rats were measured before experiment and every days through out the study. The body weight of both male and female rats were not significantly change compared to the control group. The initial weight and final weight of both male and female rats were not significant change compared to the control group. Detail was shown in **Table 7. Appendix D** were shown the body weight of both male and female rats in three months. The body weight was expressed in mean ± SD.

3.5 The internal organs weight of the rats

The internal organs weight of the male and female rats were shown in **Table 8** and **9** respectively. The lung, right testis and muscle of the male rats were significantly decreased compared to the control group ($p < 0.05$). But the right eye of male rats were significantly increased ($p < 0.05$). The heart, stomach and muscle of the female rats after exposed to carbon monoxide gas, were significantly increased ($p < 0.05$). But the left kidney of the female rats are significantly decreased ($p < 0.05$).

3.6 Hematology of the rats

The hematology results of the male and female rats were shown in **Table 10 – 11.** Hemoglobin, hematocrit, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) of the male rats, were exposed to carbon monoxide gas, were significantly increased compared to the control group ($p < 0.05$). The hemoglobin, hematocrit, MCV and MCH of the female rats, after exposed to carbon monoxide gas, were significantly increased ($p < 0.05$).

Table 7 Body weight of the rats after exposed to carbon monoxide gas.

Group		N	Initial weight (g)	Final weight (g)	% Increase
Male	Control	5	236.00 ± 14.63	430.00 ± 15.81	82.20
	CO exposure	7	242.86 ± 11.50	428.57 ± 17.96	76.47
Female	Control	6	198.33 ± 6.83285	.00 ± 15.17	43.70
	CO exposure	6	202.50 ± 4.18265	.00 ± 28.81	30.86

Table 8 The internal organs weight of the male rats after exposed to carbon monoxide gas.

Organ	Weight of internal organ (g)	
	Control	**Test**
Heart	1.505 ± 0.098	1.558 ± 0.095
Liver	15.387 ± 1.136	14.069 ± 1.156
Lung	2.730 ± 1.130	1.750 ± 0.366*
Brain	1.990 ± 0.047	1.957 ± 0.041
Kidney right	1.921 ± 0.183	1.793 ± 0.181
left	1.956 ± 0.220	1.714 ± 0.239
Adrenal gland right	0.025 ± 0.005	0.028 ± 0.003
left	0.028 ± 0.004	0.027 ± 0.003
Testis right	1.745 ± 0.126	1.323 ± 0.378*
left	1.703 ± 0.144	1.441 ± 0.311
Stomach	1.803 ± 0.201	1.766 ± 0.176
Intestinal	0.565 ± 0.088	0.636 ± 0.291
Eye right	0.127 ± 0.012	0.147 ± 0.016*
left	0.125 ± 0.010	0.141 ± 0.014
Muscle	3.936 ± 1.113	3.167 ± 0.124*
Spleen	0.859 ± 0.098	0.771 ± 0.098

* $p < 0.05$

Table 9 The internal organs weight of the female rats after exposed to carbon monoxide gas.

Organ	Weight of internal organ (g)	
	Control	**Test**
Heart	1.071 ± 0.060	1.197 ± 0.070*
Liver	10.131 ± 0.989	9.393 ± 0.976
Lung	1.789 ± 0.548	2.163 ± 0.289
Brain	1.869 ± 0.044	1.878 ± 0.156
Kidney right	1.403 ± 0.091	1.322 ± 0.118
left	1.352 ± 0.166	1.186 ± 0.130*
Adrenal gland right	0.042 ± 0.007	0.039 ± 0.006
left	0.043 ± 0.004	0.037 ± 0.005
Ovary + Uterus	1.077 ± 0.348	0.843 ± 0.121
Stomach	1.413 ± 0.078	1.681 ± 0.112*
Intestinal	0.600 ± 0.149	0.712 ± 0.104
Eye right	0.126 ± 0.015	0.124 ± 0.008
left	0.132 ± 0.015	0.136 ± 0.012
Muscle	2.782 ± 0.055	3.198 ± 0.288*
Spleen	0.756 ± 0.059	0.733 ± 0.092

* $p < 0.05$

Table 10 Hematology of the male rats after exposed to carbon monoxide gas for 3 months.

CBC	Group	
	Control	**Test**
Hemoglobin (Hb) (g/dl)	15.90 ± 0.25	18.80 ± 1.29*
Hematocrit (Hct) (%)	45.80 ± 0.84	51.43 ± 3.87*
WBC (per cu.mm.)	3,440 ± 1470.71	3,614 ± 484.52
PMN (%)	22.40 ± 4.56	22.86 ± 6.41
Lymphocyte (%)	77.60 ± 4.56	77.14 ± 6.41
RBC ($\times 10^6$ per cu.mm.)	8.21 ± 0.19	8.75 ± 0.61
MCV	55.60 ± 0.62	58.57 ± 0.70*
MCH	19.40 ± 0.42	21.50 ± 0.39*
MCHC	34.94 ± 0.50	36.73 ± 0.97*
Platelet count ($\times 10^3$ per cu.mm.)	911.20 ± 101.26	846.86 ± 88.57

* $p < 0.05$

Table 11 Hematology of the female rats after exposed to carbon monoxide gas for 3 months.

CBC	Group	
	Control	**Test**
Hemoglobin (Hb) (g/dl)	15.37 ± 0.79	18.33 ± 0.29*
Hematocrit (Hct) (%)	44.00 ± 2.53	54.50 ± 1.64*
WBC (per cu.mm.)	4,067 ± 598.89	5,583 ± 1,605.51
PMN (%)	28.00 ± 8.76	18.67 ± 6.02
Lymphocyte (%)	72.00 ± 8.76	81.33 ± 6.02
RBC ($\times 10^6$ per cu.mm.)	8.07 ± 0.62	8.36 ± 0.93
MCV	54.62 ± 1.47	59.32 ± 1.79*
MCH	19.10 ± 0.49	20.53 ± 0.76*
MCHC	34.92 ± 0.68	34.60 ± 0.91
Platelet count ($\times 10^3$ per cu.mm.)	885.33 ± 39.53	876.67 ± 28.68

* $p < 0.05$

3.7 Blood chemistry of carbon monoxide rats

Glucose, BUN, cholesterol, triglyceride, protein, bilirubin, liver enzymes and electrolytes of the carbon monoxide exposure male rats were not different from the control rats. Only creatinine was significantly increased ($p<0.05$) **(Table 12)**. In carbon monoxide exposure female rats, there were several blood chemistry determinants which were different from the control rats. Creatinine and albumin were increased but cholesterol, globulin, total bilirubin, indirect bilirubin, alkaline phosphatase and CO_2 were decreased ($p < 0.05$) **(Table 13)**.

3.8 Carboxyhemoglobin of carbon monoxide exposure rats

The carboxyhemoglobin levels in male and female carbon monoxide exposure rats were shown in **Table 14** and the calibration curve was shown in **Figure 23**. Only carboxyhemoglobin in female rats was significantly increased ($p < 0.05$). Even though the carboxyhemoglobin in male rats was also increased from the control rats but not significant.

3.9 Glutathione level of carbon monoxide exposure rats

Calibration curve of standard glutathione concentrations at 0, 10, 20, 30, 40 and 50 mg/dl versus absorbance was shown in **Figure 24**. After exposure to low concentration of carbon monoxide, the glutathione levels of both male and female rats were increased but not significantly different from the control rats **(Table 15)**. The glutathione level of both exposed carbon monoxide male and female rats were increasing but not significantly different when compared with the control group ($p < 0.05$).

3.9 Malondialdehyde of carbon monoxide exposure rats

The malondialdehyde level of the male and female rats were shown in **Table 16** and the calibration curve was shown in **Figure 25**. The malondialdehyde levels of both male and female exposed carbon monoxide rats were not significantly different from the control rats.

Table 12 Blood chemistry of the male rats after exposed to carbon monoxide gas for 3 months.

Blood chemistry	Group	
	Control	Test
Glucose (mg/dl)	156.40 ± 43.20	144.86 ± 27.88
BUN (mg/dl)	24.80 ± 3.03	24.86 ± 2.34
Creatinine (mg/dl)	0.70 ± 0.07	0.81 ± 0.14*
Cholesterol (mg/dl)	65.00 ± 12.29	70.57 ± 15.62
Triglyceride (mg/dl)	49.40 ± 14.08	55.86 ± 15.53
Total protein (g/dl)	5.16 ± 0.77	5.84 ± 0.87
Albumin (g/dl)	3.00 ± 0.50	3.39 ± 0.39
Globulin (g/dl)	2.16 ± 0.34	2.46 ± 0.50
Total bilirubin (mg/dl)	0.08 ± 0.05	0.11 ± 0.07
Direct bilirubin (mg/dl)	0.03 ± 0.02	0.03 ± 0.02
Indirect bilirubin (mg/dl)	0.05 ± 0.03	0.09 ± 0.06
AST (U/L)	110.40 ± 19.53	114.00 ± 26.50
ALT (U/L)	44.00 ± 8.54	36.86 ± 6.36
ALP (U/L)	114.2 ± 32.31	113.29 ± 26.64
Na (mEq/L)	150.60 ± 1.34	150.57 ± 3.05
K (mEq/L)	4.30 ± 0.54	4.29 ± 0.36
Cl (mEq/L)	122.80 ± 7.60	188.00 ± 5.39
CO_2 (mEq/L)	22.00 ± 3.00	22.57 ± 3.21
N	12	12

* $p < 0.05$

Table 13 Blood chemistry of the female rats after exposed to carbon monoxide gas for 3 months.

Blood chemistry	Group	
	Control	Test
Glucose (mg/dl)	123.83 ± 37.17	119.83 ± 27.90
BUN (mg/dl)	32.67 ± 5.13	33.50 ± 3.21
Creatinine (mg/dl)	0.92 ± 0.10	1.13 ± 0.16*
Cholesterol (mg/dl)	40.17 ± 9.35	30.33 ± 7.89*
Triglyceride (mg/dl)	61.50 ± 39.65	69.50 ± 14.68
Total protein (g/dl)	5.25 ± 0.95	4.43 ± 0.94
Albumin (g/dl)	1.53 ± 0.40	2.40 ± 0.61*
Globulin (g/dl)	3.72 ± 0.56	2.03 ± 0.10*
Total bilirubin (mg/dl)	0.20 ± 0.06	0.14 ± 0.02*
Direct bilirubin (mg/dl)	0.01 ± 0.01	0.01 ± 0.01
Indirect bilirubin (mg/dl)	0.20 ± 0.06	0.13 ± 0.02*
AST (U/L)	138.17 ± 29.79	134.17 ± 25.05
ALT (U/L)	38.67 ± 7.55	33.67 ± 4.67
ALP (U/L)	120.67 ± 27.16	75.83 ± 11.13*
Na (mEq/L)	154.00 ± 1.94	152.50 ± 2.74
K (mEq/L)	4.60 ± 0.74	3.80 ± 0.49
Cl (mEq/L)	123.17 ± 7.94	129.33 ± 3.88
CO_2 (mEq/L)	20.00 ± 2.10	17.00 ± 2.28*
N	12	12

* $p < 0.05$

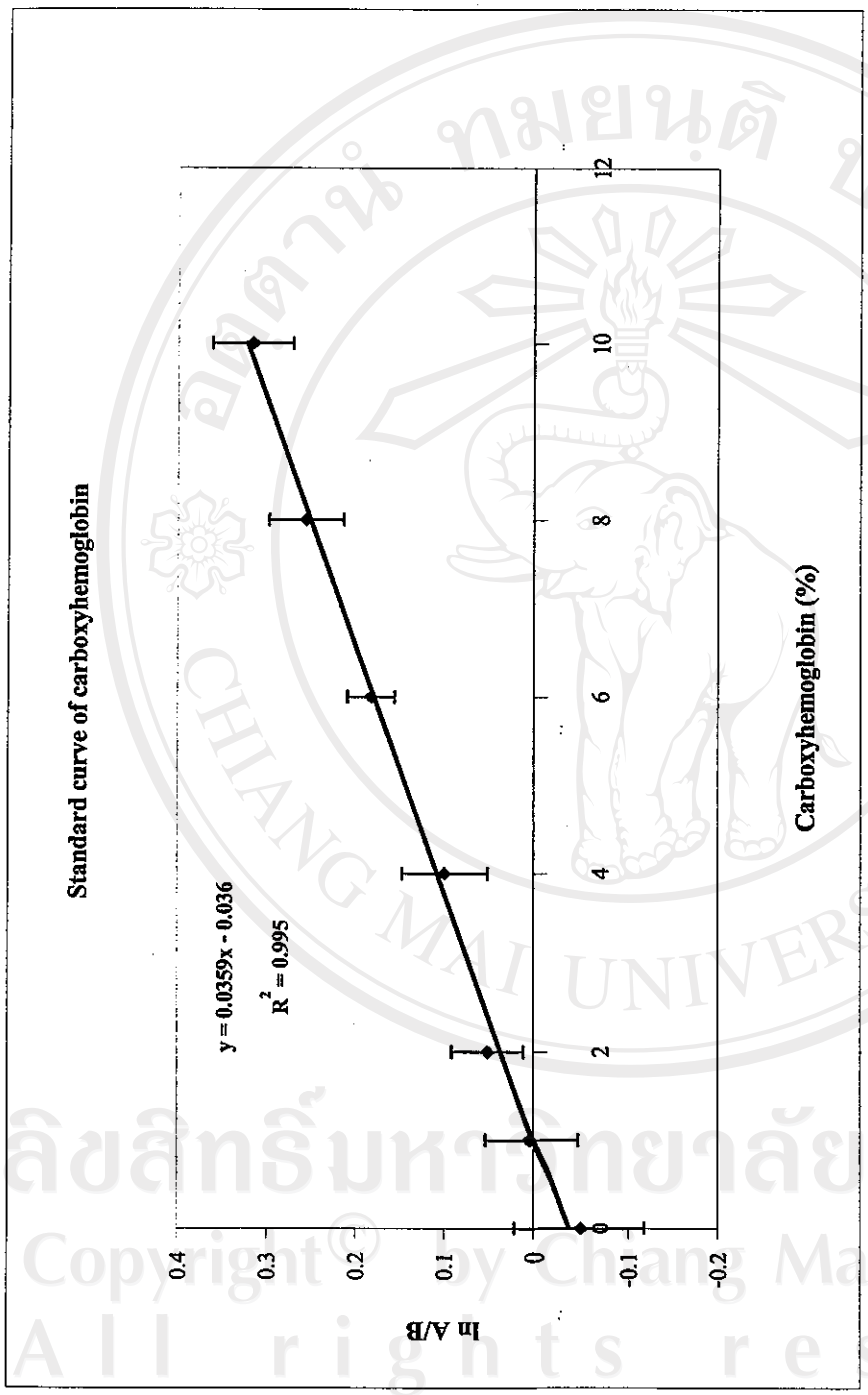

Figure 23 Calibration curve of standard carboxyhemoglobin. ln A/B is natural logarithm of ln A/B ratio ($A=A_{418} - A_{409}$; $B = A_{432} - A_{441}$ nm A_{409}, A_{418}, A_{432}, A_{441} are absorbance at 409, 418, 432 and 441 nm).

Table 14 Mean ± standard deviation of carboxyhemoglobin of male and female rats after exposure to low concentration of carbon monoxide gas for 3 months.

Rats	Carboxyhemoglobin (%)	
	Male	**Female**
Control	2.52 ± 0.51 (N = 5)	1.20 ± 0.96 (N = 6)
CO exposure	3.59 ± 1.70 (N = 7)	4.00 ± 1.80* (N = 6)

* $p < 0.05$

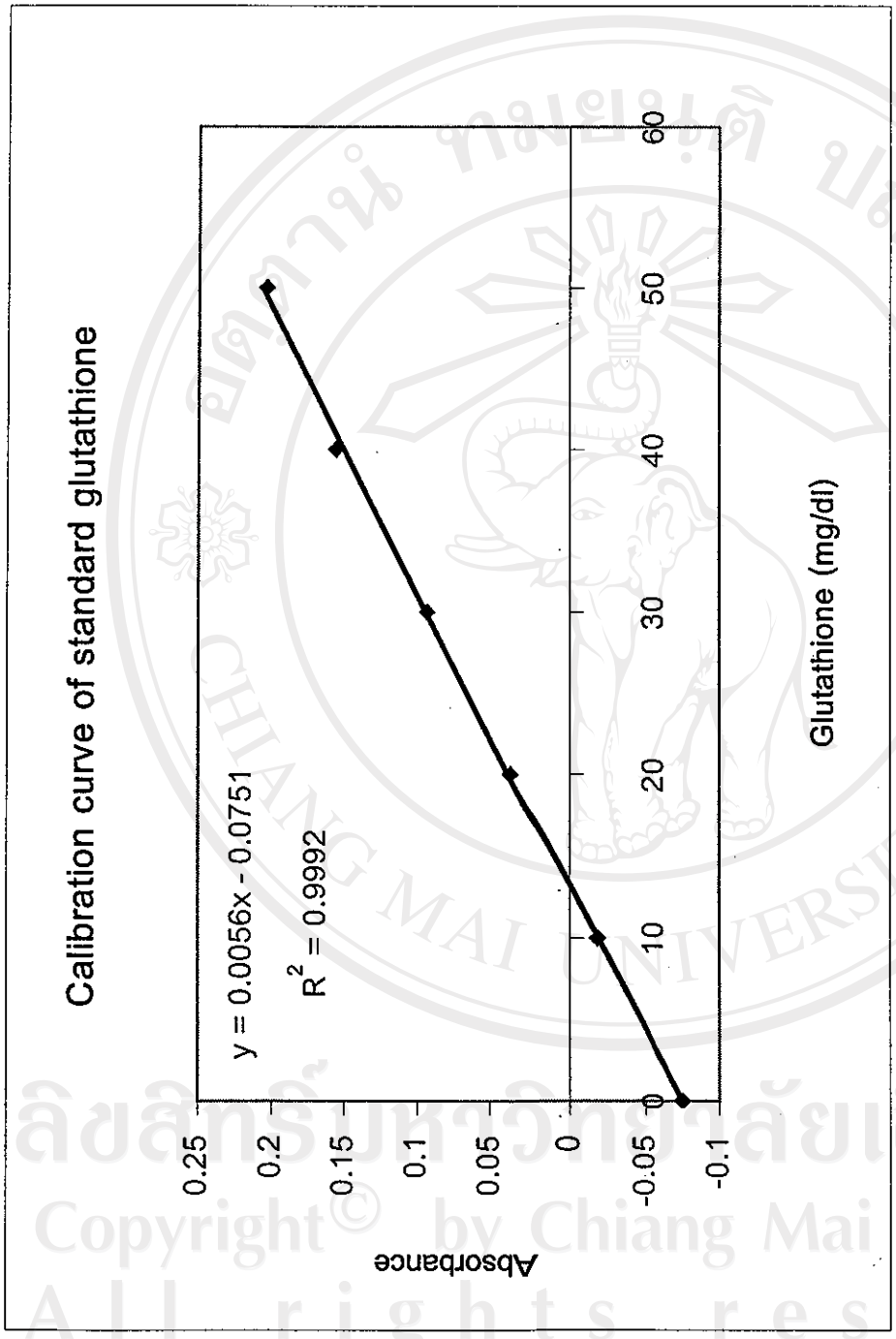

Figure 24 Calibration curve of standard glutathione.

Table 15 Mean ± standard deviation of glutathione levels in male and female rats after exposure to low concentration of carbon monoxide gas for 3 months.

Rats	Glutathione (mg/dl)	
	Male	**Female**
Control	38.18 ± 4.38 (N = 5)	43.61 ± 20.24 (N = 6)
CO exposure	41.98 ± 10.69 (N = 7)	50.37 ± 32.01 (N = 6)

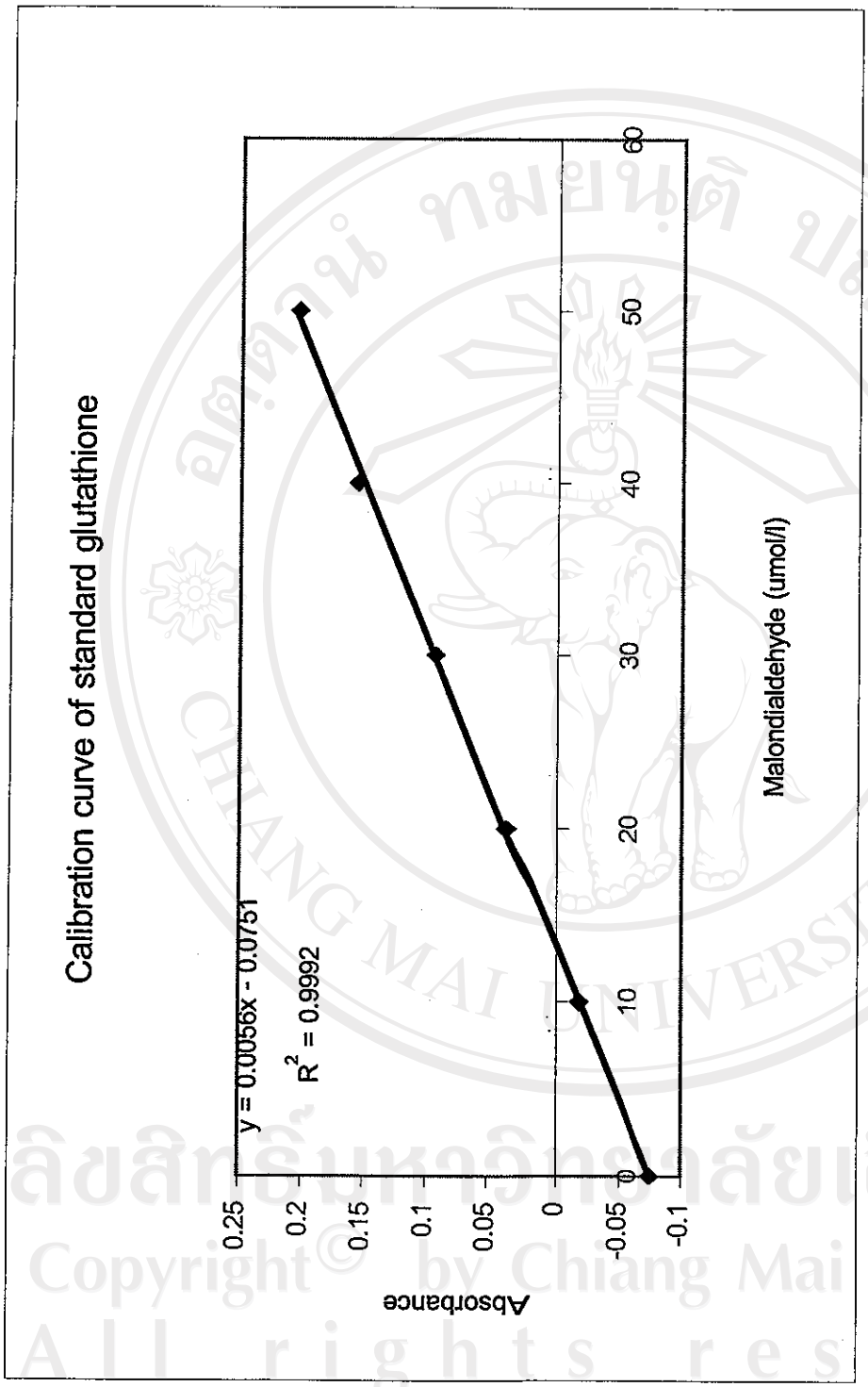

Figure 25 Calibration curv e of standard malondialdehyde

Table 16 Mean ± standard deviation of malondialdehyde levels in male and fem ale rats after exposure to low concentration of carbon monoxide gas for 3 months.

Group	Malondialdehyde (μmol/l)	
	Male	**Female**
Control	32.32 ± 7.44 (N = 5)	21.52 ± 9.54 (N = 6)
CO exposure	38.73 ± 7.78 (N = 7)	17.92 ± 7.95 (N = 6)

3.10 Cytochrome *c* reduction in carbon monoxide exposure rats

Cytochrome *c* in the brain and lung of carbon monoxide exposure rats were shown in **Table 17.** It was shown that there was no significantly decreased of the cytochrome *c* in both tissues. However, there was appeared to be lower than the cytochrome *c* in both tissues in the control rats.

Table 17 Mean ± standard deviation of cytochrome *c* levels in male and fem ale rats after exposure to low concentration of carbon monoxide gas for 3 months.

Organ	Rats	Cytochrome *c* (nmol/15 mins)	
		Male	**Female**
Brain	Control	16,149.00 ± 2,333.52 (N = 5)	14,651.70 ± 2,987.80 (N = 6)
	CO exposure	16,007.10 ± 3,197.58 (N = 7)	11,683.70 ± 728.15* (N = 6)
Lung	Control	17,284.68 ± 3,025.39 (N = 5)	14,817.95 ± 2,605.81 (N = 6)
	CO exposure	16,468.50 ± 1,479.29 (N = 7)	13,257.30 ± 937.60 (N = 6)

* $p < 0.05$