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APPENDICES

Table 7 Seven-day body weight of individual rat

Rat No.	Day 0	Day 7	Day 14	Day 21	Day 28
Control group					
1	244.0	311.7	346.0	378.0	390.4
2	263.5	295.5	322.6	340.7	353.5
3	334.5	377.6	417.5	443.2	462.6
4	300.7	345.5	368.0	382.8	395.6
5	331.0	364.5	383.0	392.5	406.5
6	296.7	321.5	344.5	360.0	373.2
7	338.0	349.0	361.8	369.0	384.1
8	389.5	411.0	433.0	444.7	457.0
9	341.0	357.0	368.6	381.0	384.8
10	285.0	288.0	305.5	321.0	339.4
Mean ± SEM	312.39 ± 13.52	342.13 ± 12.13	365.05 ± 12.41	381.29 ± 12.42	394.71 ± 12.51
HS-group I					
1	241.5	289.3	312.0	335.0	351.1
2	271.5	300.0	321.7	330.6	343.3
3	293.5	317.5	341.7	343.2	350.8
4	336.5	367.5	387.3	397.4	408.0
5	341.5	375.0	404.5	428.6	454.3
6	346.0	373.0	388.9	404.6	418.5
7	379.4	400.0	421.0	433.5	458.9
8	309.0	319.1	337.0	344.7	356.6
9	383.5	404.9	416.4	430.0	445.4
10	387.4	395.3	408.2	414.4	425.0
Mean ± SEM	328.98 ± 15.65	354.16 ± 13.76	373.87 ± 13.12	386.20 ± 13.54	401.19 ± 14.68
HS-group II					
1	293.2	321.1	357.0	382.8	397.5
2	308.5	312.0	330.0	321.3	332.0
3	280.0	295.3	313.8	324.0	333.0
4	298.5	327.0	342.5	352.5	363.0
5	300.5	315.2	320.0	329.8	341.0
6	298.3	323.0	337.6	349.4	345.5
7	311.2	329.8	336.3	353.3	362.5
8	300.8	318.5	339.1	352.6	357.8
9	-	-	-	-	-
10	350.0	365.0	375.0	377.6	380.8
Mean ± SEM	304.56 ± 6.42	322.99 ± 6.24	339.03 ± 6.14	349.25 ± 7.23	357.01 ± 7.32

Unit expressed as g.

Table 8 Terminal body weight of individual rat

Rat No.	Group		
	Control	**HS-group I**	**HS-group II**
1	376.80	337.00	383.70
2	342.60	333.10	320.00
3	441.80	319.50	318.00
4	381.20	395.00	351.40
5	389.60	432.80	327.90
6	356.50	402.30	341.10
7	364.00	435.90	346.60
8	442.30	348.10	348.00
9	375.00	428.00	-
10	333.30	410.00	367.20
Average	380.31	384.17	344.88
SEM	11.65	14.30	7.17

Unit expressed as g

Table 9 Liver weight of individual rat

Rat No.	Group		
	Control	**HS-group I**	**HS-group II**
1	10.69	9.60	10.88
2	10.89	9.89	8.66
3	13.59	9.35	11.36
4	11.12	12.96	12.17
5	12.58	12.46	12.53
6	11.25	13.94	11.20
7	12.81	14.11	8.98
8	17.28	12.25	11.83
9	11.33	16.00	13.73
10	12.26	20.04	15.36
Average	12.38	13.06	11.67
SEM	0.62	1.03	0.63

Unit expressed as g

Table 10 Food consumption of individual rat

Rat No.	Day 5	Day 10	Day 15	Day 20	Day 25
Control group					
1	24	18	20	19	17
2	24	18	20	19	17
3	19	22	23	24	18
4	19	22	23	24	18
5	20	18	23	17	17
6	20	18	23	17	17
7	20	14	17	27	20
8	20	14	17	27	20
9	33	16	19	18	16
10	33	16	19	18	16
Mean ± SEM	23.20 ± 1.73	17.60 ± 0.88	20.40 ± 0.78	21.00 ± 1.28	17.60 ± 0.45
HS-group I					
1	22	18	21	23	20
2	22	18	21	23	20
3	23	17	19	18	18
4	23	17	19	18	18
5	23	21	23	25	21
6	23	21	23	25	21
7	17	16	23	26	23
8	17	16	23	26	23
9	21	26	30	28	28
10	21	26	30	28	28
Mean ± SEM	21.20 ± 0.74	19.60 ± 1.20	23.20 ± 1.24	24.00 ± 1.14	22.00 ± 1.14
HS-group II					
1	20	22	20	18	20
2	20	22	20	18	20
3	21	25	25	19	18
4	21	25	25	19	18
5	17	18	18	20	16
6	17	18	18	20	16
7	17	15	16	22	20
8	17	15	16	22	20
9	21	19	25	-	-
10	21	19	25	21	16
Mean ± SEM	19.20 ± 0.61	19.80 ± 1.14	20.80 ± 1.22	19.89 ± 0.51	18.22 ± 0.62

Unit expressed as g/day

Table 11 Relative food consumption of individual rat

Rat No.	Day 7	Day 14	Day 21	Day 28
Control group				
1	0.077	0.058	0.050	0.044
2	0.081	0.062	0.056	0.048
3	0.050	0.055	0.054	0.039
4	0.055	0.063	0.063	0.046
5	0.055	0.060	0.043	0.042
6	0.062	0.067	0.047	0.046
7	0.057	0.047	0.073	0.052
8	0.049	0.039	0.061	0.044
9	0.092	0.052	0.047	0.042
10	0.115	0.062	0.056	0.047
Mean ± SEM	0.0693 ± 0.0068	0.0565 ± 0.0027	0.0550 ± 0.0028	0.0450 ± 0.0015
HS-group I				
1	0.076	0.067	0.069	0.057
2	0.073	0.065	0.070	0.058
3	0.072	0.056	0.052	0.051
4	0.063	0.049	0.045	0.044
5	0.061	0.057	0.058	0.046
6	0.062	0.059	0.062	0.050
7	0.043	0.055	0.060	0.050
8	0.053	0.068	0.075	0.064
9	0.052	0.072	0.065	0.063
10	0.053	0.073	0.068	0.066
Mean ± SEM	0.0608 ± 0.0034	0.0621 ± 0.0025	0.0624 ± 0.0029	0.0549 ± 0.0025
HS-group II				
1	0.062	0.056	0.047	0.050
2	0.064	0.061	0.056	0.060
3	0.071	0.080	0.059	0.054
4	0.064	0.073	0.054	0.050
5	0.054	0.056	0.061	0.047
6	0.053	0.053	0.057	0.046
7	0.052	0.048	0.062	0.055
8	0.053	0.047	0.062	0.056
9	-	-	-	-
10	0.058	0.067	0.056	0.042
Mean ± SEM	0.0590 ± 0.0022	0.0601 ± 0.0038	0.0571 ± 0.0016	0.0511 ± 0.0019

Unit expressed as g/B.W./day

Table 12 Water consumption of individual rat

Rat No.	Day 5	Day 10	Day 15	Day 20	Day 25
Control group					
1	46	35	44	36	42
2	46	35	44	36	42
3	37	46	48	50	41
4	37	46	48	50	41
5	38	52	48	51	44
6	38	52	48	51	44
7	33	29	32	39	30
8	33	29	32	39	30
9	32	24	33	30	27
10	32	24	33	30	27
Mean ± SEM	37.20 ± 1.65	37.20 ± 3.47	41.00 ± 2.37	41.20 ± 2.71	36.80 ± 2.30
HS-group I					
1	39	43	50	34	34
2	39	43	50	34	34
3	33	28	33	26	30
4	33	28	33	26	30
5	35	38	38	43	41
6	35	38	38	43	41
7	38	30	31	42	44
8	38	30	31	42	44
9	43	34	49	35	38
10	43	34	49	35	38
Mean ± SEM	37.60 ± 1.15	34.60 ± 1.81	40.20 ± 2.64	36.00 ± 2.05	37.40 ± 1.65
HS-group II					
1	31	35	38	31	36
2	31	35	38	31	36
3	34	30	33	33	36
4	34	30	33	33	36
5	34	32	32	33	29
6	34	32	32	33	29
7	29	28	29	36	32
8	29	28	29	36	32
9	33	35	45	-	-
10	33	35	45	42	35
Mean ± SEM	32.20 ± 0.65	32.00 ± 0.92	35.40 ± 1.87	34.22 ± 1.14	33.44 ± 1.00

Unit expressed as ml/day

Table 13 Relative water consumption of individual rat

Rat No.	Day 7	Day 14	Day 21	Day 28
Control group				
1	0.148	0.127	0.095	0.108
2	0.156	0.136	0.106	0.119
3	0.098	0.115	0.113	0.089
4	0.107	0.130	0.131	0.104
5	0.104	0.125	0.130	0.108
6	0.118	0.139	0.142	0.118
7	0.095	0.088	0.106	0.078
8	0.080	0.074	0.088	0.066
9	0.090	0.090	0.079	0.070
10	0.111	0.108	0.093	0.080
Mean ± SEM	0.1107 ± 0.0077	0.1132 ± 0.0071	0.1083 ± 0.0065	0.0940 ± 0.0063
HS-group I				
1	0.135	0.160	0.101	0.097
2	0.130	0.155	0.103	0.099
3	0.104	0.097	0.076	0.086
4	0.090	0.085	0.065	0.074
5	0.093	0.094	0.100	0.090
6	0.094	0.098	0.106	0.098
7	0.095	0.074	0.097	0.096
8	0.119	0.092	0.122	0.123
9	0.106	0.118	0.081	0.085
10	0.109	0.120	0.084	0.089
Mean ± SEM	0.1075 ± 0.0050	0.1093 ± 0.0091	0.0935 ± 0.0053	0.0937 ± 0.0041
HS-group II				
1	0.097	0.106	0.081	0.091
2	0.099	0.115	0.096	0.108
3	0.115	0.105	0.102	0.108
4	0.104	0.096	0.094	0.099
5	0.108	0.100	0.100	0.085
6	0.105	0.095	0.094	0.084
7	0.088	0.086	0.102	0.088
8	0.091	0.086	0.102	0.089
9	-	-	-	-
10	0.090	0.120	0.111	0.092
Mean ± SEM	0.0970 ± 0.0030	0.1010 ± 0.0039	0.0980 ± 0.0027	0.0938 ± 0.0031

Unit expressed as ml/B.W./day

Table 14 Serum ALT concentration of individual rat

Rat No.	group		
	Control	HS-group I	HS-group II
1	41	45	53
2	45	50	52
3	48	38	46
4	47	45	43
5	38	44	43
6	49	47	50
7	49	49	45
8	46	52	35
9	38	38	-
10	45	40	45
Average	44.60	44.80	45.78
SEM	1.33	1.55	1.83

Unit expressed as U/L

Table 15 Serum AST concentration of individual rat

Rat No.	Group		
	Control	HS-group I	HS-group II
1	159	208	181
2	196	212	217
3	124	162	187
4	169	172	177
5	123	189	194
6	120	137	148
7	152	189	173
8	130	187	174
9	106	131	-
10	112	115	101
Average	139.10	170.20	172.44
SEM	9.10	10.50	10.84

Unit expressed as U/L

Table 16 Serum alkaline phosphatase concentration of individual rat

Rat No.	group		
	Control	**HS-group I**	**HS-group II**
1	95	141	146
2	158	141	128
3	98	108	106
4	87	121	101
5	91	85	103
6	96	112	114
7	111	123	92
8	106	108	131
9	87	83	-
10	146	143	147
Average	107.50	116.50	118.67
SEM	7.84	6.87	6.71

Unit expressed as U/L

Table 17 Serum total bilirubin concentration of individual rat

Rat No.	group		
	Control	**HS-group I**	**HS-group II**
1	0.1	0.1	0.1
2	0.1	0.1	0.1
3	0.1	0.1	0.1
4	0.1	0.1	0.1
5	0.1	0.1	0.1
6	0.1	0.1	0.1
7	0.1	0.2	0.1
8	0.1	0.1	0.1
9	0.1	0.1	0.4
10	0.1	0.1	0.1
Average	0.10	0.11	0.13
SEM	0.00	0.01	0.03

Unit expressed as mg/dl

Table 18 Serum direct bilirubin concentration of individual rat

Rat No.	group		
	Control	HS-group I	HS-group II
1	0	0	0
2	0.1	0	0.1
3	0	0	0
4	0	0	0
5	0	0	0
6	0	0	0
7	0	0.1	0
8	0	0	0.1
9	0	0	0.2
10	0	0	0
Average	0.01	0.01	0.04
SEM	0.01	0.01	0.02

Unit expressed as mg/dl

Table 19 Serum total protein concentration of individual rat

Rat No.	group		
	Control	HS-group I	HS-group II
1	6.9	6.6	6.6
2	6.8	6.5	6.2
3	6.3	6.6	6.3
4	6.8	6.6	6.9
5	6.7	6.7	6.7
6	6.5	6.5	6.7
7	6.2	6.6	6.6
8	6.6	6.8	6.5
9	7.1	6.5	6.9
10	6.6	6.9	6.9
Average	6.65	6.63	6.63
SEM	0.09	0.04	0.08

Unit expressed as g/dl

Table 20 Serum albumin concentration of individual rat

Rat No.	group		
	Control	HS-group I	HS-group II
1	3.8	3.6	3.5
2	3.8	3.6	3.3
3	3.5	3.7	3.5
4	3.9	3.5	3.8
5	3.5	3.5	3.6
6	3.4	3.6	3.7
7	3.2	3.8	3.6
8	3.7	3.7	3.5
9	3.8	3.3	-
10	3.3	3.5	3.6
Average	3.59	3.58	3.57
SEM	0.08	0.04	0.04

Unit expressed as g/dl

Table 21 Serum globulin concentration of individual rat

Rat No.	group		
	Control	HS-group I	HS-group II
1	3.1	3.0	3.1
2	3.0	2.9	2.9
3	2.8	2.9	2.8
4	2.9	3.1	3.1
5	3.2	3.2	3.1
6	3.1	2.9	3.0
7	3.0	2.8	3.0
8	2.9	3.1	3.0
9	3.3	3.2	-
10	3.3	3.4	3.3
Average	3.06	3.05	3.03
SEM	0.05	0.06	0.05

Unit expressed as g/dl

Table 22 BUN concentration of individual rat

Rat No.	group		
	Control	HS-group I	HS-group II
1	15	15	18
2	18	16	17
3	21	21	21
4	22	16	17
5	12	16	22
6	14	16	39
7	19	18	18
8	17	21	23
9	21	15	25
10	20	23	25
Average	17.90	17.70	22.50
SEM	1.06	0.92	2.08

Unit expressed as mg/dl

Table 23 SCr concentration of individual rat

Rat No.	group		
	Control	HS-group I	HS-group II
1	0.5	0.6	0.6
2	0.6	0.6	0.5
3	0.5	0.7	0.7
4	0.8	0.6	0.6
5	0.6	0.5	0.8
6	0.5	0.6	1.1
7	0.6	0.6	0.6
8	0.6	0.7	0.6
9	0.6	0.6	0.7
10	0.6	0.6	0.6
Average	0.59	0.61	0.68
SEM	0.03	0.02	0.05

Unit expressed as mg/dl

Table 24 Serum total cholesterol concentration of individual rat

Rat No.	group		
	Control	HS-group I	HS-group II
1	74	65	62
2	88	64	61
3	61	60	51
4	68	58	60
5	79	79	51
6	82	71	61
7	59	63	74
8	60	82	82
9	68	49	61
10	68	59	68
Average	70.70	65.00	63.10
SEM	3.12	3.15	3.01

Unit expressed as mg/dl

Table 25 Serum TG concentration of individual rat

Rat No.	group		
	Control	HS-group I	HS-group II
1	92	123	148
2	93	90	64
3	118	85	73
4	78	91	119
5	80	95	54
6	148	106	68
7	109	110	77
8	148	99	122
9	79	113	100
10	80	241	100
Average	102.50	115.30	92.50
SEM	8.68	14.45	9.59

Unit expressed as mg/dl

Table 26 Serum LDL-C concentration of individual rat

Rat No.	group		
	Control	HS-group I	HS-group II
1	2	3	1
2	4	4	6
3	1	3	3
4	4	2	2
5	5	2	5
6	2	2	4
7	2	1	4
8	1	4	5
9	6	1	-
10	6	1	4
Average	3.30	2.30	3.78
SEM	0.62	0.36	0.52

Unit expressed as mg/dl

Table 27 Serum HDL-C concentration of individual rat

Rat No.	group		
	Control	HS-group I	HS-group II
1	63	57	55
2	74	56	54
3	58	55	50
4	66	57	56
5	70	72	49
6	71	65	55
7	54	57	65
8	54	73	69
9	61	45	-
10	60	47	59
Average	63.10	58.40	56.89
SEM	2.22	2.93	2.18

Unit expressed as mg/dl

Table 28 Serum glucose concentration of individual rat

Rat No.	group		
	Control	HS-group I	HS-group II
1	123	107	110
2	107	103	88
3	113	87	90
4	84	106	95
5	102	95	78
6	128	107	91
7	105	94	105
8	111	112	107
9	95	118	-
10	113	126	112
Average	108.10	105.50	97.33
SEM	4.05	3.67	3.89

Unit expressed as mg/dl

Table 29 Serum uric acid concentration of individual rat

Rat No.	group		
	Control	HS-group I	HS-group II
1	1.2	1.3	1.0
2	1.4	1.2	1.2
3	1.0	0.8	0.7
4	0.9	0.9	0.8
5	0.7	0.6	0.6
6	1.1	1.1	1.2
7	0.6	1.2	1.2
8	1.0	1.9	1.4
9	1.5	1.6	1.3
10	1.1	1.0	1.5
Average	1.05	1.16	1.09
SEM	0.09	0.12	0.09

Unit expressed as mg/dl

Table 30 Serum calcium concentration of individual rat

Rat No.	group		
	Control	HS-group I	HS-group II
1	10.0	10.1	9.7
2	10.7	10.1	10.5
3	10.3	10.4	10.1
4	9.6	10.1	10.3
5	9.8	10.3	10.1
6	9.7	9.9	9.9
7	9.5	10.5	9.7
8	9.9	10.1	10.0
9	10.2	9.9	10.2
10	9.6	10.1	9.7
Average	9.93	10.15	10.02
SEM	0.12	0.06	0.09

Unit expressed as mg/dl

Table 31 Serum sodium concentration of individual rat

Rat No.	group		
	Control	HS-group I	HS-group II
1	151	149	151
2	161	160	159
3	149	152	150
4	150	150	150
5	150	151	151
6	150	148	150
7	145	152	148
8	150	150	151
9	143	142	152
10	145	147	146
Average	149.40	150.10	150.80
SEM	1.56	1.44	1.06

Unit expressed as mEq/L

Table 32 Serum potassium concentration of individual rat

Rat No.	group		
	Control	HS-group I	HS-group II
1	4.5	4.7	4.1
2	5.4	4.4	5.1
3	4.7	4.1	4.4
4	4.1	4.7	4.2
5	3.9	3.9	4.6
6	4.4	3.8	5.0
7	4.4	4.9	4.1
8	3.9	4.7	4.3
9	4.5	4.1	4.7
10	4.5	5.8	5.7
Average	4.43	4.51	4.62
SEM	0.14	0.19	0.16

Unit expressed as mEq/L

Table 33 Serum chloride concentration of individual rat

Rat No.	Group		
	Control	HS-group I	HS-group II
1	105	109	107
2	115	113	114
3	107	106	107
4	104	105	104
5	105	107	106
6	108	107	105
7	106	109	108
8	108	104	109
9	102	104	106
10	104	106	106
Average	106.40	107.00	107.20
SEM	1.13	0.87	0.88

Unit expressed as mEq/L

Table 34 Hb of individual rat

Rat No.	group		
	Control	HS-group I	HS-group II
1	14.5	13.5	14.5
2	14.2	18.5	15.1
3	13.9	14.4	16.0
4	17.4	14.7	13.3
5	14.4	14.8	15.7
6	14.3	15.0	13.4
7	14.6	15.4	15.0
8	15.2	13.8	13.7
9	16.6	12.3	16.0
10	15.8	14.9	15.8
Average	15.09	14.73	14.85
SEM	0.37	0.51	0.34

Unit expressed as g/dl

Table 35 Hct of individual rat

Rat No.	group		
	Control	HS-group I	HS-group II
1	46	44	46
2	44	56	46
3	44	46	51
4	55	45	41
5	44	47	49
6	44	49	43
7	46	51	47
8	46	49	43
9	51	39	50
10	50	46	49
Average	47.00	47.20	46.50
SEM	1.19	1.43	1.06

Unit expressed as %

Table 36 RBC count of individual rat

Rat No.	group		
	Control	**HS-group I**	**HS-group II**
1	8.10	7.84	7.94
2	7.98	9.70	8.18
3	7.98	8.24	9.22
4	9.50	7.98	7.74
5	7.74	8.46	9.16
6	8.10	8.70	7.76
7	7.34	8.18	7.70
8	8.46	7.54	7.30
9	9.50	7.06	8.44
10	9.00	8.56	9.18
Average	8.37	8.23	8.26
SEM	0.23	0.23	0.22

Unit expressed as million cells/cumm

Table 37 RBC indices (MCV, MCH and MCHC)

Rat No.	MCV (fL)	MCH (pg)	MCHC (g/dl)
Control			
1	56.4	17.9	31.7
2	55.5	17.8	32.1
3	55.0	17.5	31.8
4	57.6	18.3	31.8
5	57.5	18.6	32.4
6	54.0	17.7	32.7
7	62.9	19.9	31.6
8	54.8	18.0	32.8
9	53.2	17.5	32.9
10	56.0	17.6	31.5
Mean ± SEM	56.29 ± 0.86	18.08 ± 0.22	32.13 ± 0.17
HS-group I			
1	55.7	17.2	31.0
2	57.3	19.0	33.2
3	55.7	17.5	31.3
4	56.6	18.5	32.6
5	55.6	17.5	31.4
6	56.5	17.2	30.5
7	61.9	18.8	30.4
8	65.0	18.3	28.2
9	55.6	17.4	31.4
10	53.8	17.4	32.3
Mean ± SEM	57.37 ± 1.08	17.88 ± 0.22	31.23 ± 0.44
HS-group II			
1	57.9	18.3	31.6
2	56.3	18.5	32.8
3	55.5	17.4	31.3
4	53.0	17.1	32.3
5	53.6	17.1	31.8
6	55.2	17.3	31.3
7	60.5	19.5	32.2
8	58.4	18.1	31.0
9	58.8	19.0	32.3
10	53.8	17.2	32.0
Mean ± SEM	56.30 ± 0.80	17.95 ± 0.27	31.86 ± 0.18

Table 38 Platelet count of individual rat

Rat No.	group		
	Control	HS-group I	HS-group II
1	682,000	840,000	628,000
2	618,000	1,150,000	742,000
3	846,000	816,000	910,000
4	860,000	888,000	658,000
5	796,000	746,000	710,000
6	784,000	796,000	750,000
7	650,000	794,000	736,000
8	486,000	802,000	710,000
9	1,112,000	480,000	-
10	716,000	802,000	882,000
Average	755,000.00	811,400.00	727,000.00
SEM	53,570.51	51,226.77	26,726.12

Unit expressed as cells/cumm

Table 39 WBC count of individual rat

Rat No.	group		
	Control	HS-group I	HS-group II
1	492	1,610	2,620
2	2,440	3,580	2,280
3	1,742	1,192	2,260
4	1,804	1,976	1,756
5	1,900	1,042	1,240
6	906	718	450
7	2,560	2,480	1,520
8	3,060	1,380	1,140
9	2,500	840	-
10	1,334	504	1,412
Average	1,873.80	1,532.20	1,630.89
SEM	252.78	295.77	225.45

Unit expressed as cells/cumm

Table 40 RBC morphology of individual rat

Rat No.	group		
	Control	**HS-group I**	**HS-group II**
1	normal	normal	normal
2	normal	normal	normal
3	normal	normal	normal
4	normal	normal	normal
5	normal	normal	normal
6	normal	normal	normal
7	normal	normal	normal
8	normal	normal	normal
9	normal	normal	normal
10	normal	normal	normal

Table 41 Percent differential WBCs of individual rat

Rat No.	% differential WBCs				
	PMN	Lymphocyte	Monocyte	Eosinophil	Basophil
Control					
1	11	84	2	3	0
2	22	72	2	4	0
3	27	70	2	1	0
4	17	80	3	0	0
5	28	67	5	0	0
6	40	57	1	2	0
7	28	68	3	1	0
8	10	77	2	1	0
9	27	70	2	1	0
10	26	70	4	0	0
Mean ± SEM	23.60 ± 2.84	71.50 ± 2.38	2.60 ± 0.37	1.30 ± 0.42	0
HS-group I					
1	23	74	3	0	0
2	11	85	2	2	0
3	19	80	1	0	0
4	22	74	4	0	0
5	35	62	3	0	0
6	17	80	2	1	0
7	23	75	2	0	0
8	26	70	3	1	0
9	21	77	2	0	0
10	63	36	1	0	0
Mean ± SEM	26.00 ± 4.55	71.30 ± 4.39	2.30 ± 0.30	0.40 ± 0.22	0
HS-group II					
1	24	75	0	1	0
2	36	60	1	2	1
3	25	72	3	0	0
4	22	74	4	0	0
5	17	82	1	0	0
6	20	73	5	2	0
7	22	74	2	2	0
8	30	67	2	1	0
9	-	-	-	-	-
10	24	75	1	0	0
Mean ± SEM	24.44 ± 1.87	72.44 ± 2.01	2.11 ± 0.54	0.89 ± 0.31	0.11 ± 0.11

Unit expressed as %

Table 42 Microsomal protein concentration of individual rat

Rat No.	Group		
	Control	HS-group I	HS-group II
1	29.26	22.50	15.56
2	30.28	28.61	16.30
3	25.56	26.39	18.52
4	35.65	38.06	18.61
5	18.65	41.45	17.41
6	38.35	37.43	32.06
7	23.81	55.07	30.10
8	36.91	29.28	38.77
9	41.55	36.91	33.20
10	41.14	56.82	50.32
Average	32.12	37.25	27.09
SEM	2.47	3.64	3.71

Unit expressed as mg/ml

Table 43 Hepatic microsomal total CYP content of individual rat

Rat No.		Group	
	Control	HS-group I	HS-group II
1	0.50	0.77	0.75
2	0.73	0.63	0.74
3	0.56	0.68	0.45
4	0.62	0.47	0.58
5	0.62	0.36	0.43
6	0.42	0.45	0.46
7	0.67	0.41	0.45
8	0.51	0.52	0.45
9	0.45	0.54	-
10	0.48	0.31	0.39
Average	0.556	0.514	0.522
SEM	0.032	0.046	0.045

Unit expressed as nmol/mg protein

Table 44 Hepatic microsomal EROD activity of individual rat

Rat No.	group		
	Control	HS-group I	HS-group II
1	11	21	17
2	15	12	15
3	24	13	8
4	11	11	9
5	11	22	11
6	32	49	75
7	56	31	29
8	28	17	38
9	34	32	-
10	54	38	51
Average	27.60	24.60	28.11
SEM	5.34	3.98	7.62

Unit expressed as pmol/mg protein/min

Table 45 Hepatic microsomal MROD activity of individual rat

Rat No.	group		
	Control	HS-group I	HS-group II
1	3	5	7
2	13	9	15
3	7	10	7
4	7	9	8
5	8	7	10
6	9	6	14
7	11	7	7
8	7	5	9
9	7	7	-
10	10	5	8
Average	8.20	7.00	9.44
SEM	0.87	0.58	1.02

Unit expressed as pmol/mg protein/min

Table 46 Hepatic microsomal BROD activity of individual rat

Rat No.	group		
	Control	HS-group I	HS-group II
1	8	36	33
2	32	22	15
3	13	28	22
4	21	19	15
5	23	16	25
6	16	24	43
7	37	25	24
8	20	12	26
9	16	14	-
10	29	18	25
Average	21.50	21.40	25.33
SEM	2.84	2.28	2.88

Unit expressed as pmol/mg protein/min

Table 47 Hepatic microsomal PROD activity of individual rat

Rat No.		group	
	Control	HS-group I	HS-group II
1	6	9	12
2	2	2	2
3	2	2	6
4	2	1	5
5	1	7	4
6	3	6	12
7	11	6	7
8	7	4	11
9	5	8	-
10	9	6	11
Average	4.80	5.10	7.78
SEM	1.07	0.86	1.27

Unit expressed as pmol/mg protein/min

Table 48 Hepatic microsomal aniline 4-hydroxylase activity of individual rat

Rat No.	group		
	Control	HS-group I	HS-group II
1	0.158	0.175	0.166
2	0.119	0.103	0.179
3	0.065	0.149	0.086
4	0.052	0.053	0.053
5	0.127	0.056	0.114
6	0.069	0.067	0.114
7	0.092	0.042	0.064
8	0.042	0.056	0.058
9	0.051	0.050	-
10	0.051	0.057	0.065
Average	0.083	0.081	0.100
SEM	0.013	0.015	0.016

Unit expressed as nmol/mg protein/min

Table 49 Hepatic microsomal erythromycin N-demethylase activity of individual rat

Rat No.	group		
	Control	HS-group I	HS-group II
1	0.673	0.911	1.022
2	0.683	0.769	0.936
3	1.042	0.936	1.081
4	0.891	0.880	1.014
5	0.863	0.644	0.594
6	0.880	0.683	0.700
7	1.073	0.908	0.988
8	1.138	0.847	0.945
9	1.267	1.130	-
10	1.226	0.567	0.671
Average	0.974	0.828	0.883
SEM	0.066	0.052	0.060

Unit expressed as nmol/mg protein/min

Table 50 Normal values of hematology and clinical blood chemistry parameters in rat (Coxgad, S., and Chenglis, C.P., 1992; Harkness, J.E., and Wagner, J.E., 1995; Olferd, E.D., Cross, B.M., and William, A.M., 1993)

Blood parameters	Normal values
ALT (U/L)	25-55
AST (U/L)	60-300
ALP (U/L)	56.8-128
Total bilirubin (mg/dl)	0-0.55
Total protein (g/dl)	5.6-7.6
Albumin (g/dl)	3.8-4.8
Globulin (g/dl)	1.8-3.0
BUN (mg/dl)	5-29
SCr (mg/dl)	0.2-0.8
Total cholesterol (mg/dl)	40-130
Triglycerides (mg/dl)	26-145
Glucose (mg/dl)	50-135
Uric acid (mg/dl)	1.2-7.5
Calcium (mg/dl)	5.3-13.0
Sodium (mEq/L)	143-156
Potassium (mEq/L)	5.4-7.0
Chloride (mEq/L)	100-110
Hb (g/dl)	11-18
Hct (%)	36-48
RBC (x 10^6 cells/mm^3)	7.2-9.6
MCV (fL)	57-65
MCH (pg)	14.6-21.3
MCHC (g/dl)	26-38
Platelet (x 10^3 cells/mm^3)	500-1,300
Neutrophil (%)	9-34
Eosinophil (%)	0-6
Basophil (%)	0-1.5
Lymphocyte (%)	65-85
Monocyte (%)	0-5

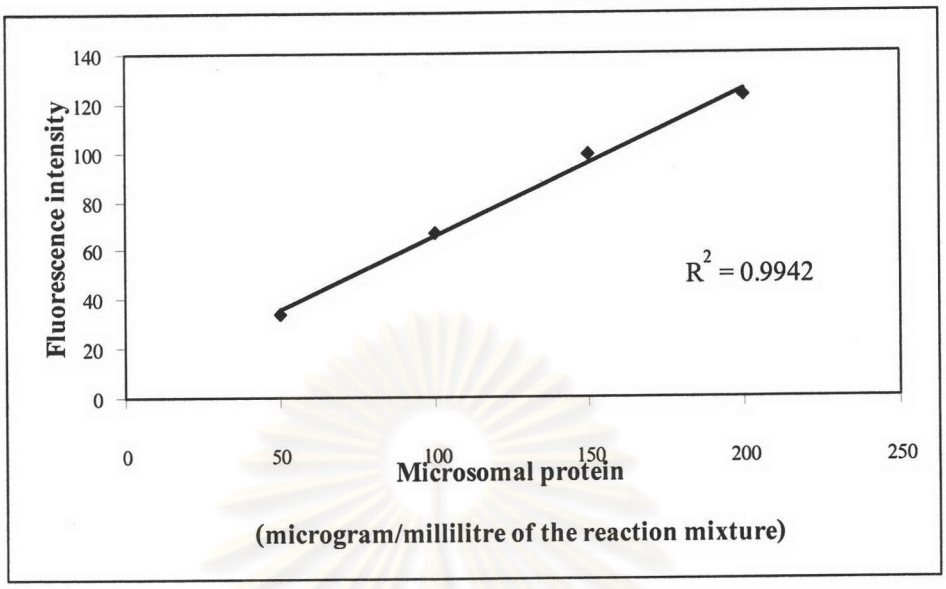

Figure 31 Verification of alkoxyresorufin O-dealkylation.

Correlation between the amount of microsomal protein used in the reaction and fluorescence intensity was shown with a correlation coefficient (r^2) of 0.9942. Each point was mean of n = 2. (Procedure was demonstrated in the Materials and Methods).

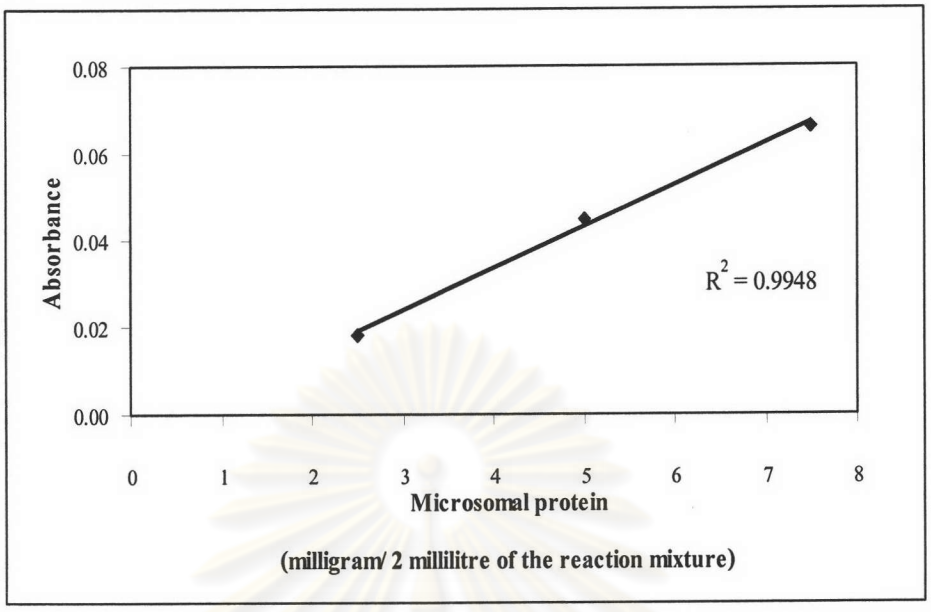

Figure 32 Verification of aniline 4-hydroxylation.

Correlation between the amount of microsomal protein used in the reaction and absorbance was shown with a correlation coefficient (r^2) of 0.9948. Each point was mean of n = 2. (Procedure was demonstrated in the Materials and Methods).

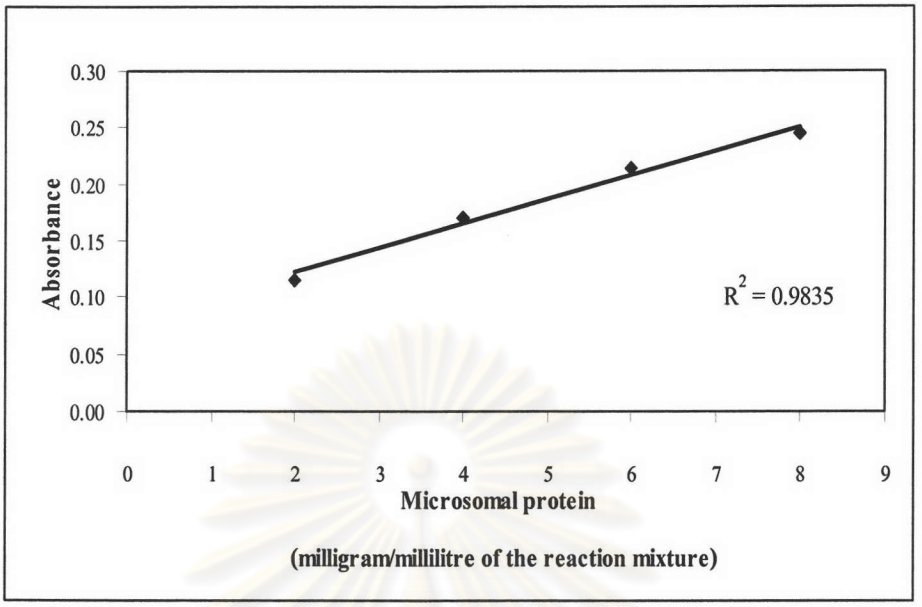

Figure 33 Verification of erythromycin N-demethylation.

Correlation between the amount of microsomal protein used in the reaction and absorbance was shown with a correlation coefficient (r^2) of 0.9835. Each point was mean of n = 2. (Procedure was demonstrated in the Materials and Methods).

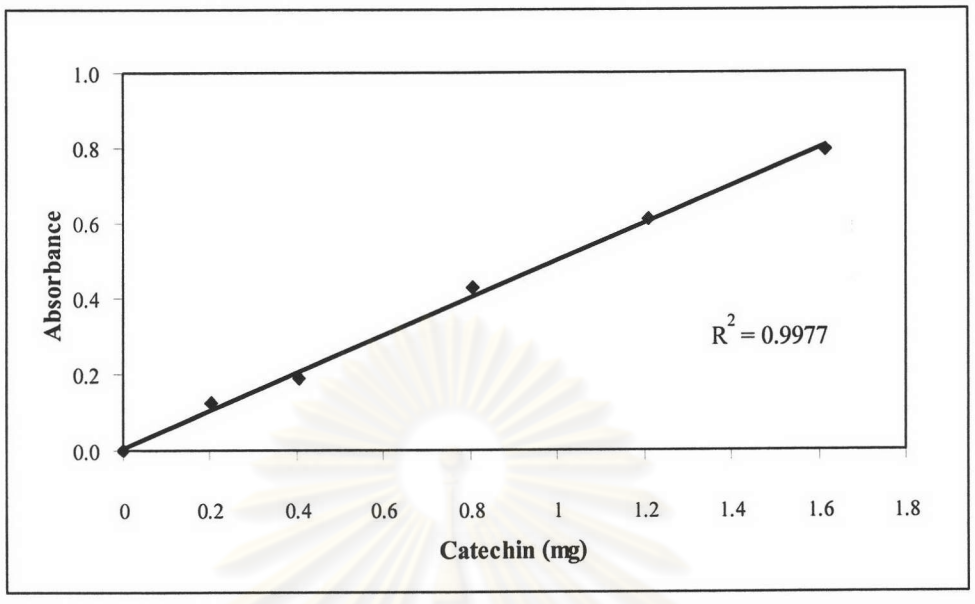

Figure 34 Standard curve of catechin which was used for the determination of total phenolic compounds.

Correlation between the amount of catechin used in the reaction and the corresponding absorbance was shown with a correlation coefficient (r^2) of 0.9977 and the regression of $y = 0.4911x + 0.0122$ (where y was absorbance, x was amount (mg) of catechin). Each point was mean of n = 2. (Procedure was demonstrated in the Materials and Methods).

Determination of total phenolic compounds in *H. sabdariffa*

Stock solution of standard catechin was prepared by dissolving 100.9 mg of catechin in 5 ml of methanol, resulting in a solution containing 20.18 mg/ml of catechin. Catechin standard curve was constructed between amount of catechin (0.2, 0.4, 0.8, 1.2, 1.6 mg) used and their corresponding absorbance. The correlation coefficient (r^2) of the standard curve was 0.9977 with the regression equation of $y = 0.4911x + 0.0122$.

The aqueous extract of *H. sabdariffa* was dissolved with ultrapure water to a concentration of 301.09 mg/ml. Then, 100 µl of the solutions used in the reaction and measured spectrometrically as described in the Materials and Methods, absorbance shown to be 0.585. Amount of total phenolic compounds in *H. sabdariffa* was calculated from the equation, $y = 0.4911x + 0.0122$.

Then, amount of total phenolic compounds in *H. sabdariffa* = (Abs-0.0122)/0.4911

= 1.1664 mg

100 µl of the solutions of *H. sabdariffa* contained amount of total phenolic compounds

= 1.1664 mg

1,000 µl of the solutions of *H. sabdariffa* contained amount of total phenolic compounds

= 11.664 mg (0.011664 g)

301.09 mg of crude extract of *H. sabdariffa* contained amount of total phenolic compounds

= 11.664 mg (0.011664 g)

So, percentage of total phenolic compounds in aqueous extract of *H. sabdariffa* was (11.664 x 100)/301.09 = 3.874 % w/w.

Because, percentage yield of extract was 24.74 % w/w. Then, the percentage of total phenolic compounds in dried calyx of *H. sabdariffa* was shown to be 0.958 % w/w.

NO. 51/ 2005

Study Protocol Approval

The Ethics Committee of the Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok, Thailand has approved the following study to be carried out according to the protocol dated and/ or amended as follows :

Study Title : SUBACUTE EFFECTS OF *HIBISCUS SABDARIFFA* AQUEOUS EXTRACT ON HEPATIC CYTOCHROME P450 AND CLINICAL BLOOD CHEMISTRY IN RATS

Study Code : -

Centre : Chulalongkorn University

Principal Investigator : MISS PROMPHORN PROMMETTA

Protocol Date : February 21, 2005

A list of the Ethics Committee members and positions present at the Ethics Committee meeting on the date of approval of this study has been attached.

This Study Protocol Approval Form will be forwarded to the Principal Investigator.

Chairman of Ethics Committee : Boonyong Tantisira
(Boonyong Tantisira, Ph.D.)

Secretary of Ethics Committee : S. Vadcharavivad
(Somratai Vadcharavivad, Pharm.D.)

Date of Approval : February 21, 2005

VITAE

Miss Promphorn Prommetta was born in January 1, 1974 in Chaiyaphum, Thailand. She graduated with Bachelor of Sciences in Pharmacy in 1997 from the Faculty of Pharmaceutical Sciences, Mahidol University, Bangkok, Thailand. After graduation, she worked as a pharmacist in Phukhieo Hospital, Chaiyaphum for six years.