CHAPTER III
RESULTS

Results are shown in two main categories, efficacies of chitosan in rats and of green tea extract in mice.

3.1 Hypolipidemic effect of the chitosan on pre-fatten rats

It is proposed that chitosan, a biopolymer of glucosamine, would utilize its cationic amino groups to electrostatically interact with negatively charge parts of such dietary lipids as derived free fatty acids and intact triglyceride molecules. Orlistat, a lipase inhibitor compound, was used as a reference hypolipidemic drug. It will interfere digestion and absorption of the exogenous lipids. At beginning, baseline levels of plasma cholesterol and triglyceride were 124±11 and 115±13 mg/dl, respectively **(Table 3.1, Table 3.2, Figure 3.1 and Figure 3.2)**. Having been fed with normal-fat diet (NF) for 12 weeks, their plasma triglyceride concentrations were a bit increased (5±7 mg/dl) and plasma total cholesterol concentrations were also increased slightly (45±22 mg/dl). Tested chitosan decreased the plasma triglyceride concentration (Δ = -20±9 mg/dl) and cholesterol concentrations (Δ = -2±26 mg/dl) of the NF-fed rats insignificantly.

Expectedly, high-fat (HF) diet markedly increased plasma cholesterol concentrations (Δ = 28±19 mg/dl) and plasma triglyceride concentrations (Δ = 35±35 mg/dl). Orlistat was effective in reducing levels of plasma triglyceride and cholesterol (Δ = -6±27 and -6±16 mg/dl). Interestingly, the chitosan was able to reduce the plasma triglyceride concentrations (Δ = -23±28 mg/dl) and cholesterol concentrations (Δ = -7±20 mg/dl) when compared to the untreated rats. The results demonstrated that the chitosan could bind electrostatically to, or trap intact triglyceride and released free fatty acid molecules, leading to interference of lipid absorption, suggesting that chitosan be a hypolipidemic factor.

Table 3-1 Plasma cholesterol and triglyceride of NF- and HF-fed rats treated with cornstarch, chitosan and orlistat. Data are shown as individual and mean±SD values.

Treatment	No.	NF diet		HF diet	
		Plasma cholesterol (mg/dl)	Plasma triglyceride (mg/dl)	Plasma cholesterol (mg/dl)	Plasma triglyceride (mg/dl)
Baseline	1	116	159	116	159
	2	108	116	108	116
	3	154	110	154	110
	4	94	77	94	77
	5	146	115	146	115
	Mean±SEM	124±11	115±13	124±11	115±13
Cornstarch	1	146	158	82	69
	2	154	100	132	205
	3	138	97	144	127
	4	144	101	186	116
	5	162	112	176	274
	6	272	92	196	108
	Mean±SEM	169±21	110±10	153±17	150±31
Chitosan	1	150	143	178	130
	2	170	124	148	88
	3	192	143	150	130
	4	184	141	172	102
	5	162	114	130	180
	6	146	113	178	130
	Mean±SEM	167±7	130±6	156±8	126±13
Orlistat	1	-	-	114	109
	2	-	-	182	182
	3	-	-	144	140
	Mean±SEM	-	-	147±20	144±21

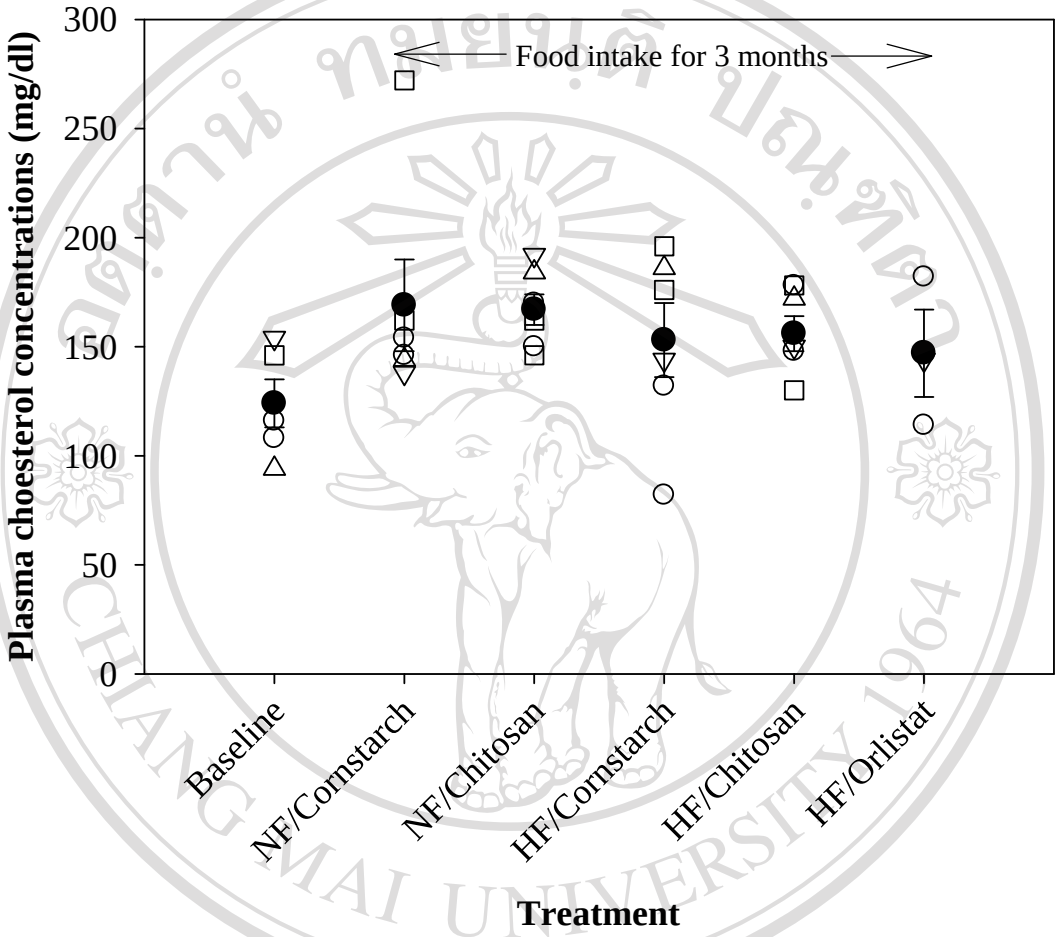


Figure 3-1 Levels of plasma cholesterol of the NF diet- and HF diet-fed rats which were treated with cornstarch, chitosan and orlistat for 3 months. Data are expressed as individual and mean±SD values.

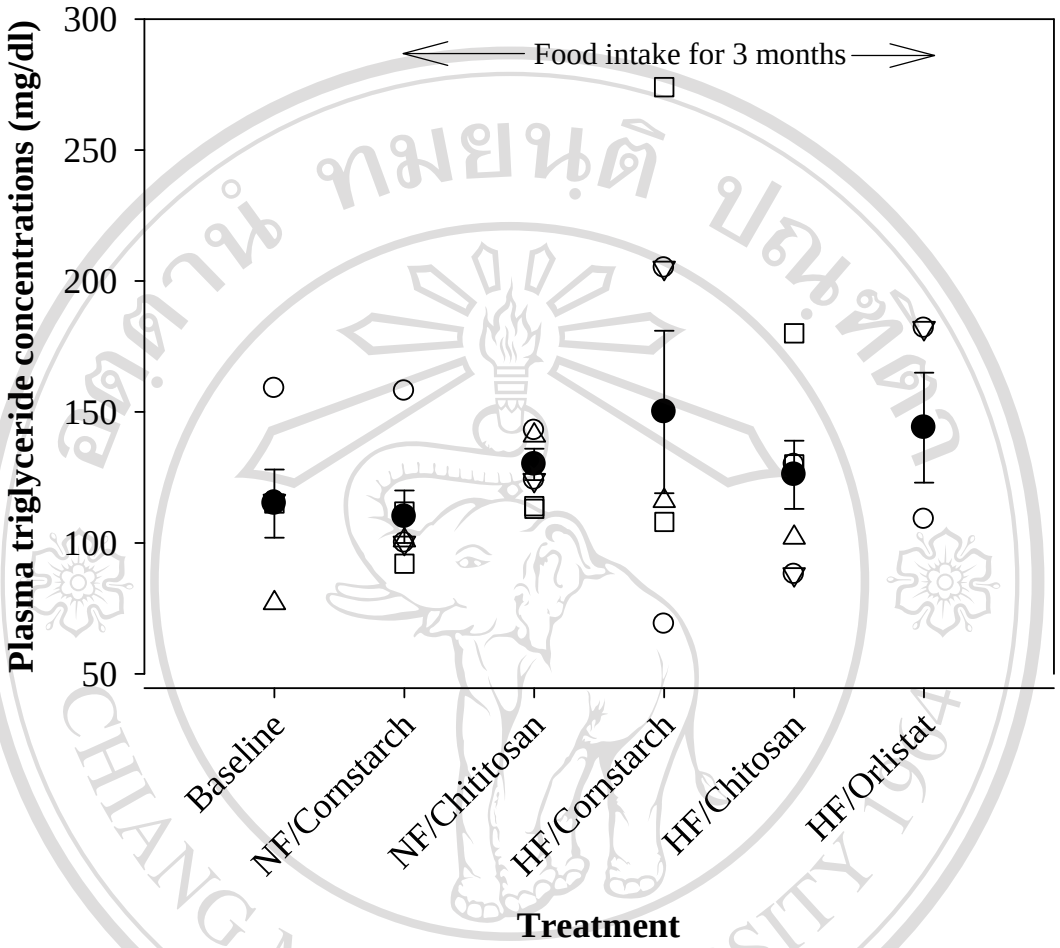


Figure 3-2 Levels of plasma triglyceride of the NF diet- and HF diet-fed rats which were treated with cornstarch, chitosan and orlistat for 3 months. Data are expressed as individual and mean±SD values.

3.2 Effects of chitosan on liver triglyceride concentrations

The NF- and HF-diet also increased liver triglyceride levels (3.38±0.52 and 5.57±0.53 mg/mg dry weight, respectively) of the rats when compared to their baseline levels (1.94±0.30 mg/mg dry weight) significantly (p <0.05) (**Table 3.2** and **Figure 3.3)**. Chitosan treatment was able to inhibit a progressive increase of liver triglyceride (2.14±0.37 mg/mg dry weight) of the NF-fed rats when compared to the placebo-treated rats (3.38±0.52 mg/mg dry weight) (Δ = -1.24±0.15 mg/mg dry weight). Chitosan and orlistat lowered triglyceride concentrations of the HF-fed rats significantly (Δ = 1.75±0.14 and 1.49±0.24 mg/mg dry weight, respectively; p <0.05) significantly. The result infers that the hypolipidemic chitosan lowered plasma triglyceride concentrations effectively, leading to lowering liver triglyceride concentration.

3.3 Effects of chitosan on liver iron status

Study rats

Clearly, the NF- and HF-diet increased liver ferritin concentrations (8.19±2.70 and 19.97±8.42 ng/mg protein, respectively) of the rats when compared to their baseline levels (3.33±1.07 ng/mg dry weight) (**Table 3.3** and **Figure 3.4)**. Chitosan brought the liver ferritin concentrations to 4.90±1.26 ng/mg protein (Δ = -4.86±1.63 ng/mg protein). Incredibly, chitosan and orlistat two-fold decreased the liver ferritin concentrations (from 19.97±8.42 ng/mg protein to 9.66±3.04 and 9.28±3.49 ng/mg protein, respectively). The data demonstrated that the chitosan and orlistat would be effective in lowering the ferritin concentrations of the rats fed with NF diet and in particular HF diet.

Table 3-2 Liver triglyceride concentrations of NF- and HF-fed rats treated with cornstarch, chitosan and orlistat. Data are expressed as individual and mean±SD values. $^{*}p$ <0.05 when compared to baseline, $^{\#}p$ <0.05 when compared to cornstarch.

Treatment	No.	NF	HF
		Liver triglyceride (mg/mg dry weight)	Liver triglyceride (mg/mg dry weight)
Baseline	1	2.11	2.11
	2	1.29	1.29
	3	1.23	1.23
	4	2.85	2.85
	5	2.22	2.22
	Mean±SEM	1.94±0.30	1.94±0.30
Cornstarch	1	5.47	3.63
	2	1.55	6.31
	3	3.15	5.47
	4	3.10	6.31
	5	3.75	6.13
	6	3.29	3.63
	Mean±SEM	3.38±0.52*	5.57±0.53*
Chitosan	1	1.23	3.98
	2	3.79	3.40
	3	2.27	5.34
	4	2.19	3.73
	5	1.34	2.40
	6	2.01	4.06
	Mean±SEM	2.14±0.37	3.82±0.39$^{\#}$
Orlistat	1	-	4.52
	2	-	3.53
	3	-	4.18
	Mean±SEM	-	4.08±0.29$^{\#}$

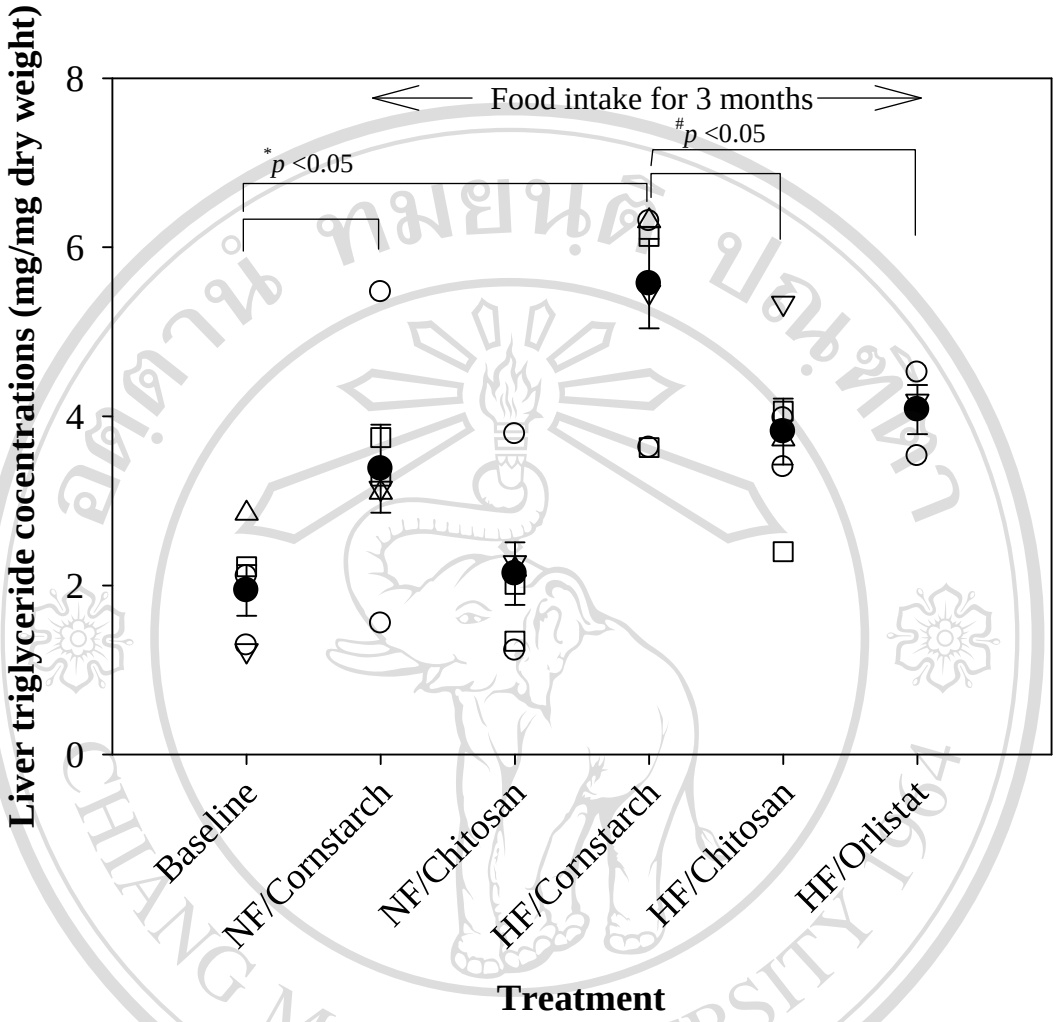


Figure 3-3 Levels of liver triglyceride of the NF diet- and HF diet-fed rats which were treated with cornstarch, chitosan and orlistat for 3 months. Data are expressed as individual and mean±SD values. $^{*}p$ <0.05 when compared to baseline, $^{\#}p$ <0.05 when compared to cornstarch.

Table 3-3 Liver ferritin of NF- and HF-fed rats treated with cornstarch, chitosan and orlistat. Data are expressed as individual and mean±SD values.

Treatment	No.	NF diet	HF diet
		Liver ferritin content (ng/ mg protein)	Liver ferritin content (ng/ mg protein)
Baseline	1	0.00	0.00
	2	0.00	0.00
	3	5.83	5.83
	4	2.24	2.24
	5	1.93	1.93
	Mean±SEM	3.33±1.07	3.33±1.07
Cornstarch	1	8.95	4.62
	2	10.79	12.98
	3	2.38	0.00
	4	17.11	2.25
	5	1.71	25.92
	6	0.00	54.05
	Mean±SEM	8.19±2.70	19.97±8.42
Chitosan	1	8.47	5.12
	2	2.25	21.80
	3	0.00	6.59
	4	4.76	5.13
	5	2.56	0.00
	6	6.46	5.12
	Mean±SEM	4.90±1.26	9.66±3.04
Orlistat	1	-	3.86
	2	-	8.18
	3	-	15.80
	Mean±SEM	-	9.28±3.49

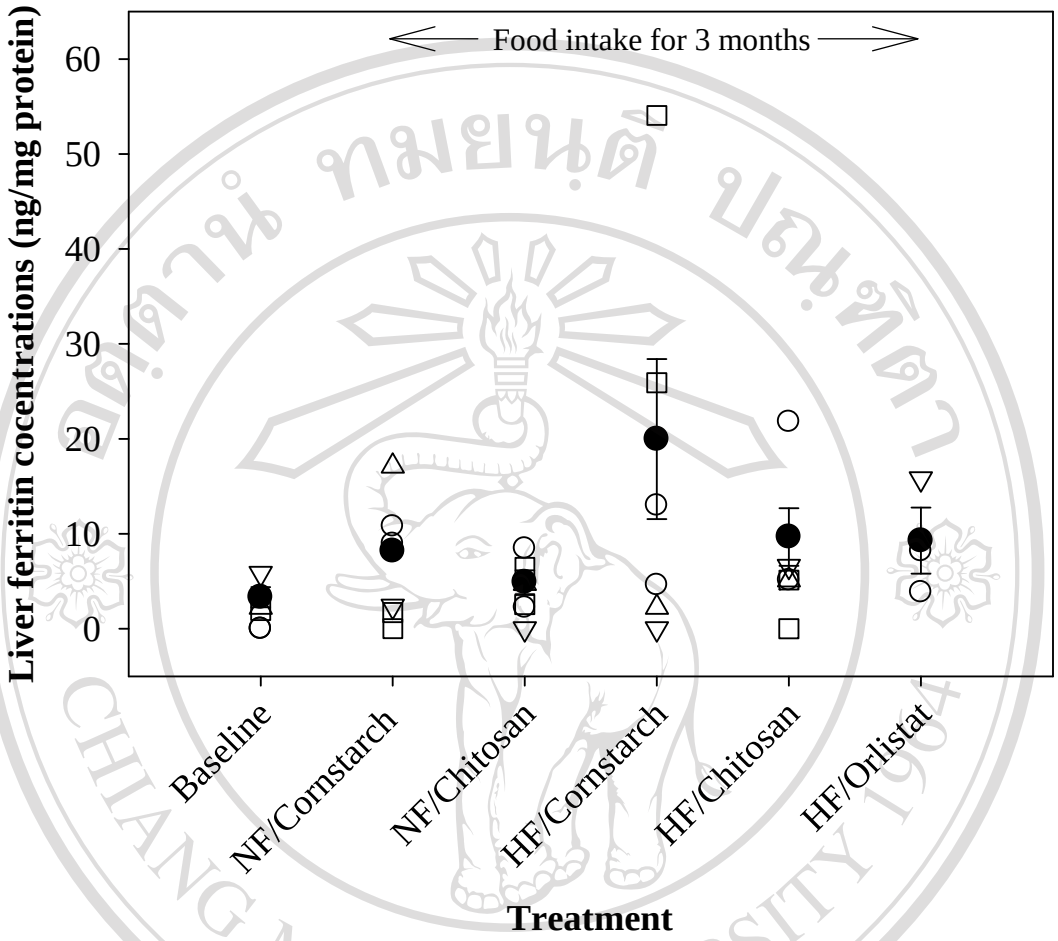


Figure 3-4 Levels of liver ferritin of the NF diet- and HF diet-fed rats which were treated with cornstarch, chitosan and orlistat for 3 months. Data are expressed as individual and mean±SD values.

As mentioned before, iron is a Fenton catalyst in generation of ROS and is stored intracellularly as ferric oxyhydroxide in iron-storage protein ferritin and its degradation product hemosiderin. High accumulation of ferritin and iron in the liver can cause oxidative stress in hepatic parenchymal and stellate cells. Our previous study has shown that hepatic enlargement and overweight are observed in the Fe diet-fed mice (unpublished data). Therefore, determination of rat and mouse LIC was performed and normalized in both mg iron/mg protein and mg iron/mg dry weight. As shown in **Table 3.4, Figure 3.5A** and **Figure 3.5B** NF and HF diets significantly decreased levels of LIC of the normal rats (1.049$\pm$0.141 mg Fe/mg protein, 11.03$\pm$1.12 ng Fe/mg dry weight) when compared to baseline levels (1.459$\pm$0.116 mg Fe/mg protein, 14.88$\pm$1.09 ng Fe/mg dry weight). Surprisingly, chitosan increased the LIC levels of the NF-fed rats (1.434$\pm$0.151 mg Fe/mg protein, 14.27$\pm$0.91 ng Fe/mg dry weight) and of the HF-fed rats (1.434$\pm$0.151 mg Fe/mg protein, 14.27$\pm$0.91 ng Fe/mg dry weight) (p <0.05). Orlistat did the same.

Table 3-4 Liver iron concentration (LIC) of NF- and HF-fed rats treated with cornstarch, chitosan and orlistat. Data are expressed as individual and mean±SD values. $^{*}p$ <0.05 when compared to baseline, $^{\#}p$ <0.05 when compared to cornstarch.

Treatment	No.	NF diet		HF diet	
		LIC (mg/mg protein)	LIC (ng/mg dry wt.)	LIC (mg/mg protein)	LIC(ng/mg dry wt.)
Baseline	1	1.569	14.85	1.569	14.85
	2	1.611	16.37	1.611	16.37
	3	1.024	10.86	1.024	10.86
	4	1.670	17.14	1.670	17.14
	5	1.420	15.16	1.420	15.16
	Mean±SEM	1.459±0.116	14.88±1.09	1.459±0.116	14.88±1.09
Cornstarch	1	0.815	9.19	0.256	3.25
	2	0.850	9.75	0.758	5.67
	3	0.595	7.16	0.642	6.93
	4	1.459	13.99	0.750	7.50
	5	1.200	12.49	0.696	7.26
	6	1.377	13.59	0.618	6.49
	Mean±SEM	1.049±0.141*	11.03±1.12*	0.620±0.076*	6.18±0.64*
Chitosan	1	1.142	11.91	0.684	7.24
	2	1.276	13.17	0.661	7.63
	3	0.917	11.76	0.736	7.87
	4	1.753	16.52	0.712	7.79
	5	1.763	16.41	0.907	9.859
	6	1.751	15.83	0.684	7.24
	Mean±SEM	1.434±0.151$^{\#}$	14.27±0.91$^{\#}$	0.731±0.037$^{\#}$	7.94±0.40$^{\#}$
Orlistat	1	-	-	1.018	10.95
	2	-	-	1.001	10.06
	3	-	-	0.912	10.10
	Mean±SEM	-	-	0.977±0.037$^{\#}$	10.37±0.3$^{\#}$

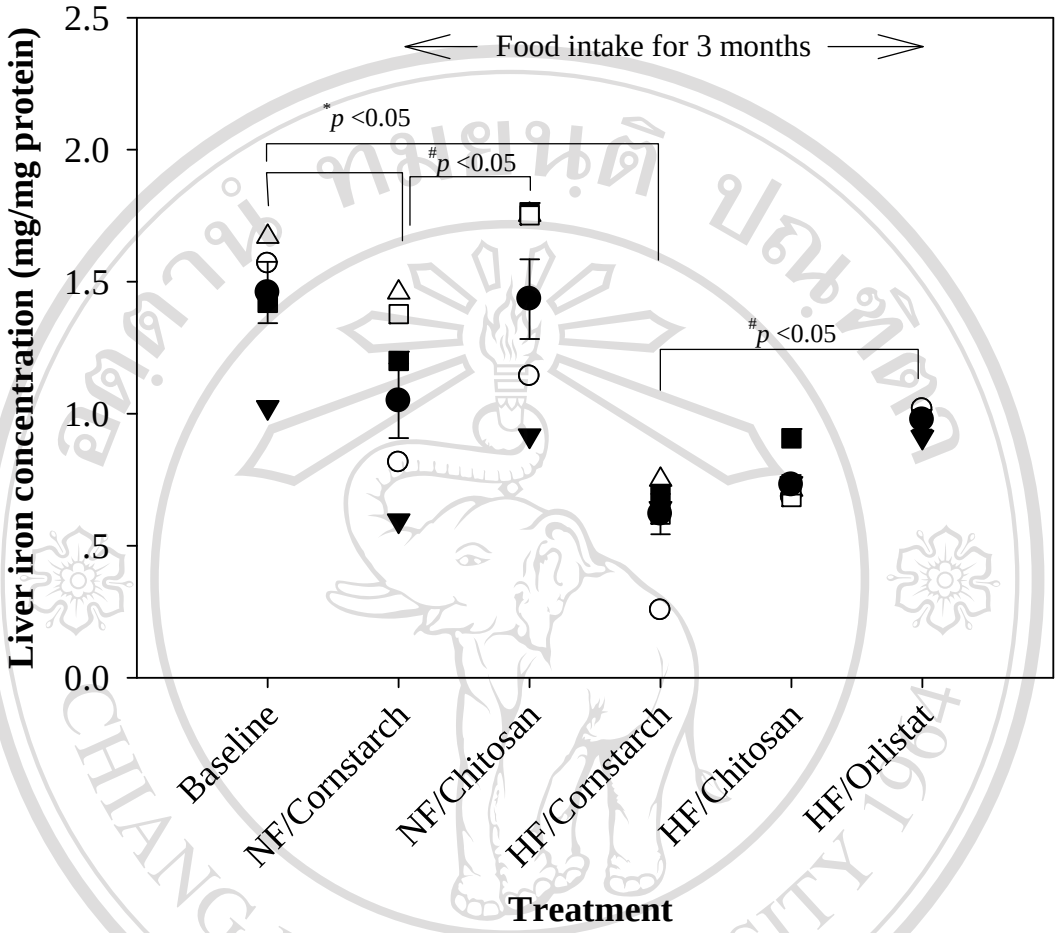


Figure 3-5A Liver iron content (LIC) of the NF diet-fed rats which were treated with cornstarch, chitosan and orlistat for 3 months. Data are expressed as individual and mean±SD values. $^{*}p$ <0.05 when compared to baseline, $^{\#}p$ <0.05 when compared to cornstarch.

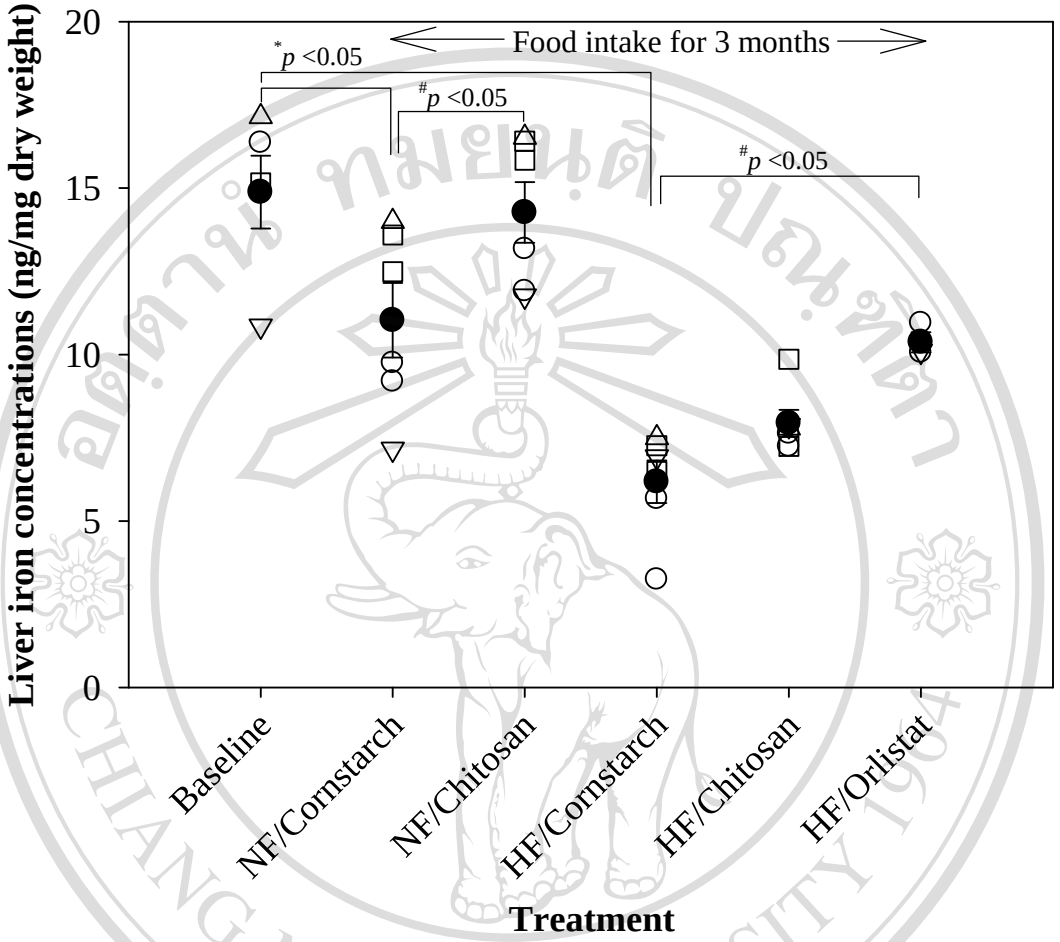


Figure 3-5B Liver iron content (LIC) of NF- and HF-fed rats which were treated with cornstarch, chitosan and orlistat for 3 months. Data are expressed as individual and mean±SD values. $^{*}p$ <0.05 when compared to baseline, $^{\#}p$ <0.05 when compared to cornstarch.

Study mice

Fe diet increased LIC levels of WT mice significantly **(**Δ = 1.209±0.578 μg Fe/mg protein, 29.13±14.44 ng Fe/ mg dry weight). GTE was effective in reducing the LIC levels **(**Δ = -1.114±0.606 μg Fe/mg protein, -27.28±15.15 ng Fe/ mg dry weight) (p <0.05), and DFP was also effective **(**Δ = -0.946±0.537 μg Fe/mg protein, -23.80±0.61 ng Fe/ mg dry weight) (**Table 3.5** and **Figure 3.6**).

Expectedly, GTE and DFP decreased the LIC levels of Fe diet-fed BKO thalassemic mice effectively and significantly (Δ = -1.615±0.895 μg Fe/mg protein by GTE, and -1.355±0.999 μg Fe/mg protein by DFP; p <0.05) (**Table 3.7, Figure 3.6** and **Figure 3.7**). The result indicated that GTE and DFP exhibited iron-chelating activities that were able to remove iron deposits in livers of the WT and BKO thalassemic mice.

Table 3-5 Liver iron concentration (LIC) of wild type (WT) mice fed with Fe diets together with oral administration (gavaging) of PBS (placebo), GTE and DFP for 2 months. Data are expressed as individual and mean±SD values.

Mice	Treatment	No.	Liver iron concentration	
			μg Fe/mg protein	ng Fe/ mg dry weight
WT	Normal diet	1	0.333	6.30
		2	0.623	11.64
		3	0.448	8.52
		4	0.613	10.25
		Mean±SEM	0.504±0.070	9.18±1.15
WT	Fe diet/Placebo	1	2.784	62.82
		2	2.883	67.67
		3	0.505	10.03
		4	0.681	12.73
		Mean±SEM	1.713±0.648*	38.31±15.59*
WT	Fe diet/GTE	1	0.700	12.15
		2	0.616	11.04
		3	0.581	10.96
		4	0.498	9.97
		Mean±SEM	0.599±0.042#	11.03±0.44#
WT	Fe diet/DFP	1	0.693	12.79
		2	0.511	9.82
		3	1.038	18.57
		4	0.827	16.87
		Mean±SEM	0.767±0.111	14.51±14.98

Table 3-6 Liver iron concentration (LIC) of heterozygous β-knockout (BKO) mice fed with Fe diets together with oral administration (gavaging) of PBS (placebo), GTE and DFP for 2 months. Data are expressed as individual and mean±SD values. *p <0.05 when compared to Fe diet/Placebo.

Mice	**Treatment**	**No.**	**Liver iron concentration**	
			μg Fe/mg protein	ng Fe/ mg dry weight
BKO	Fe diet/Placebo	1	0.901	17.81
		2	3.000	66.71
		Mean±SEM	1.950±1.049	42.26±24.45
BKO	Fe diet/GTE	1	0.450	9.81
		2	0.719	17.25
		3	0.084	1.81
		4	0.088	1.74
		Mean±SEM	0.335±0.154#	7.65±3.72#
BKO	Fe diet/DFP	1	0.109	1.98
		2	0.009	0.18
		Mean±SEM	0.595±0.050#	1.08±0.90#

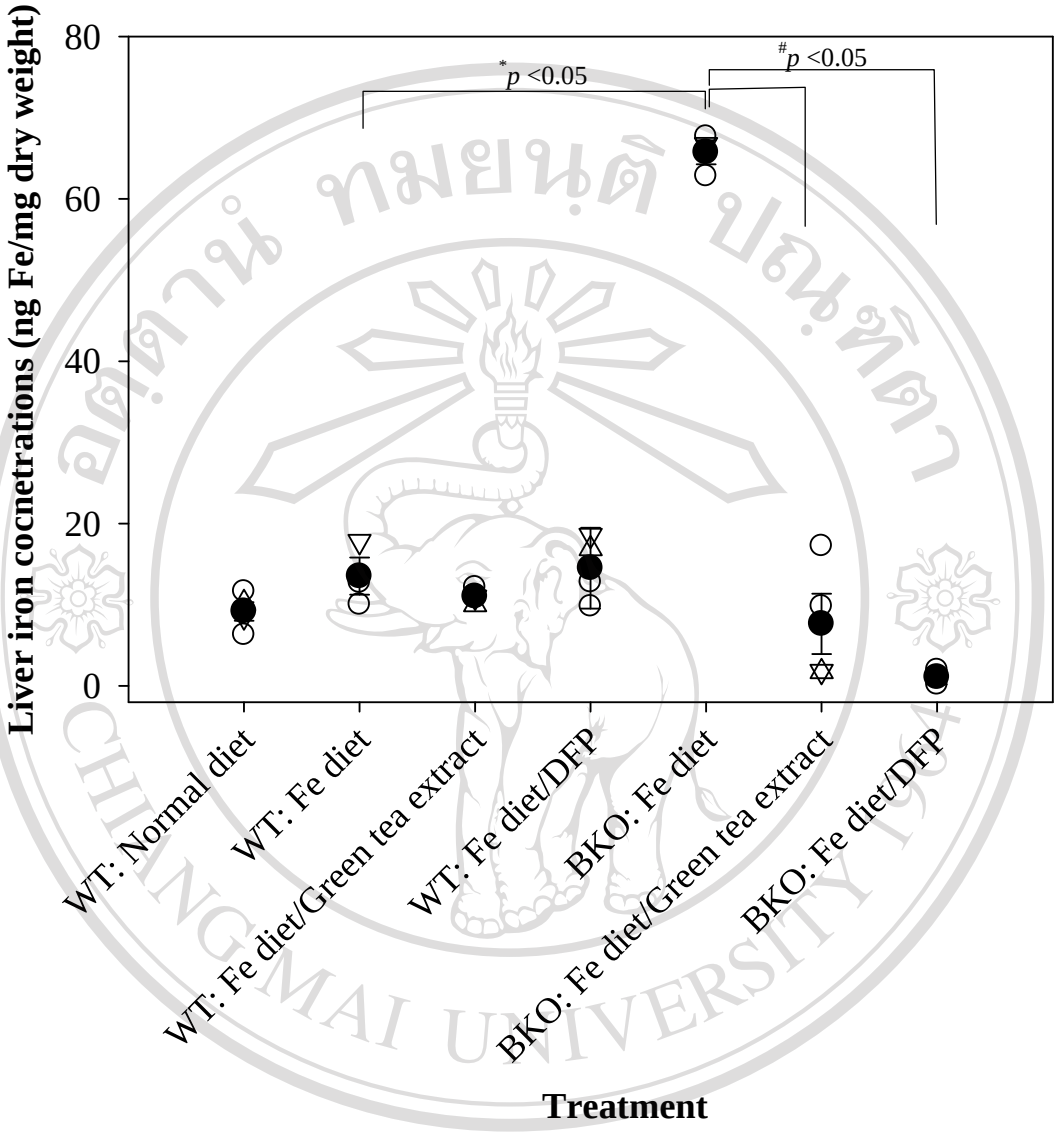


Figure 3-6 Liver iron concentration (LIC) of the wild type (WT) and heterozygous β-knockout (BKO) mice fed with Fe diets together with oral administration (gavaging) of PBS (placebo), GTE and DFP for 2 months. Data are expressed as individual and mean±SD values. $^{\#}p$ <0.05 when compared to Fe diet/Placebo.

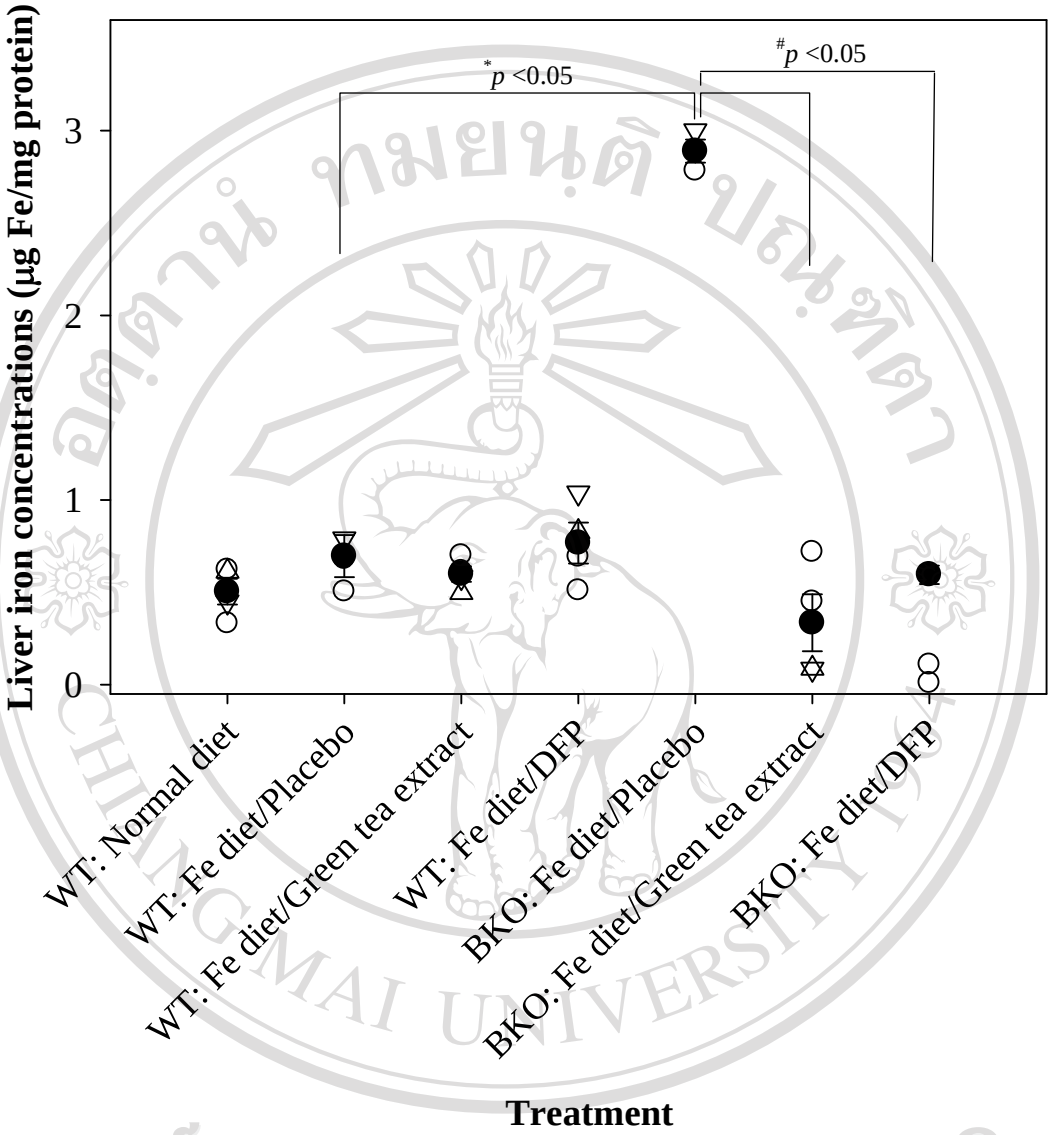


Figure 3.7 Liver iron concentration (LIC) of the wild type (WT) and heterozygous β-knockout (BKO) mice fed with Fe diets together with oral administration (gavaging) of PBS (placebo), GTE and DFP for 2 months. Data are expressed as individual and mean±SD values. $^{\#}p$ <0.05 when compared to Fe diet/Placebo.

3.4 Effects of chitosan on liver glutathione and collagen contents

Table 3-7A Liver glutathione (GSH) and collagen concentrations of NF- and HF-fed rats treated with cornstarch, chitosan and orlistat. Data are expressed as individual and mean±SD values (nmol/mg protein). $^{*}p$ <0.05 when compared to baseline.

Treatment	No.	NF diet		HF diet	
		GSH	collagen	GSH	collagen
Baseline	1	ND	0.174	ND	0.174
	2	1.04	0.268	1.04	0.268
	3	1.26	0.220	1.26	0.220
	4	0.83	0.241	0.83	0.241
	5	1.23	0.254	1.23	0.254
	Mean±SEM	1.09±0.10	0.232±0.016	1.09±0.10	0.232±0.016
Cornstarch	1	1.17	0.129	1.15	0.112
	2	1.45	0.076	1.54	0.195
	3	1.93	0.173	ND	0.091
	4	1.60	0.197	1.17	0.141
	5	ND	0.225	2.22	0.141
	6	0.94	0.241	1.24	0.257
	Mean±SEM	1.42±0.17*	0.174±0.025*	1.41±0.17*	0.156±0.025*
Chitosan	1	1.05	0.129	1.38	0.170
	2	2.22	0.263	1.28	0.259
	3	1.49	0.166	1.15	0.163
	4	1.82	0.276	1.35	0.225
	5	1.02	0.174	ND	0.258
	6	1.13	0.181	ND	0.170
	Mean±SEM	1.45±0.20	0.198±0.024	1.29±0.05	0.215±0.018
Orlistat	1	-	-	1.22	0.182
	2	-	-	1.39	0.078
	3	-	-	1.21	0.211
	Mean±SEM	-	-	1.27±0.06	0.157±0.040

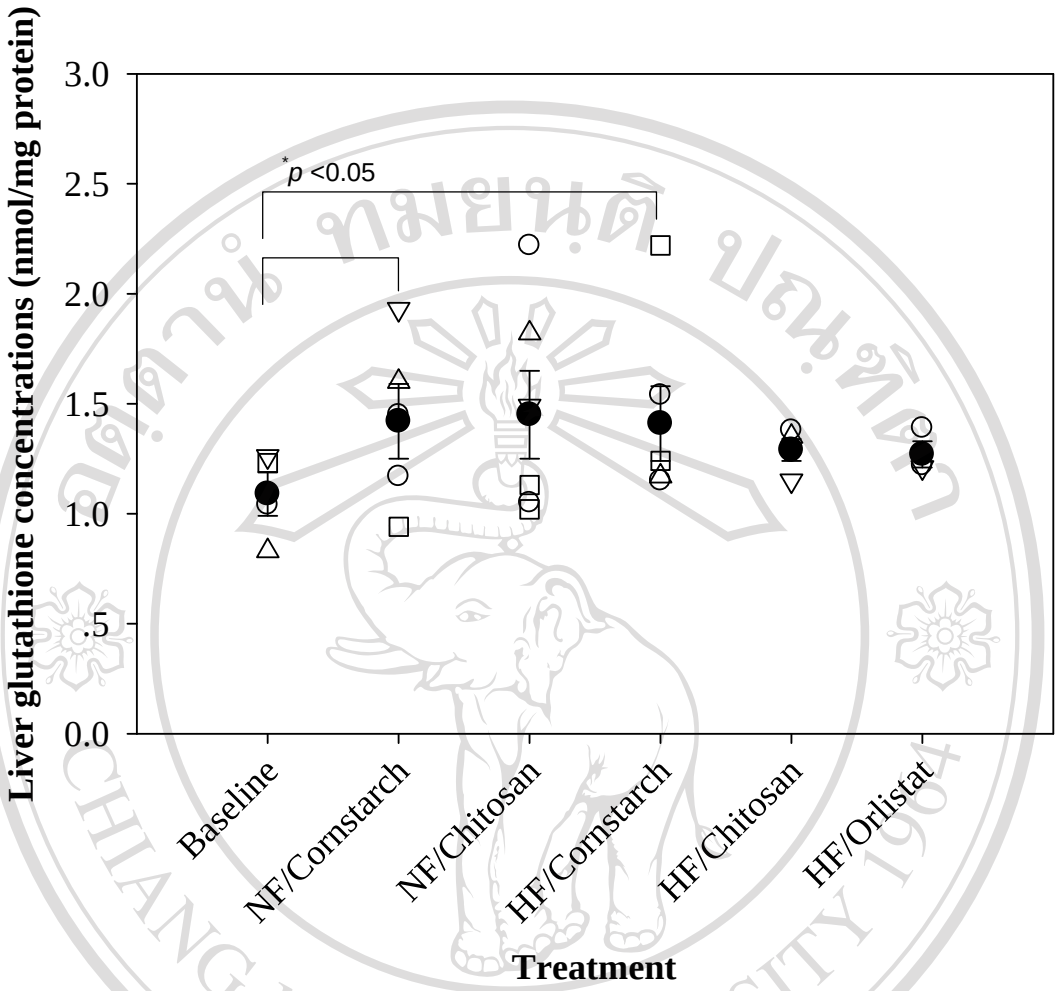


Figure 3-8 Liver glutathione (GSH) concentrations of the NF diet- and HF diet-fed rats which were treated with cornstarch, chitosan and orlistat for 3 months. Data are expressed as individual and mean±SD values.

Study in rats

While growing up for 3 months, amounts of glutathione (reduced form of glutathione, GSH) were added up in livers of the NF-fed and HF-fed rats (from 1.09±0.10 nmol/mg protein to 1.42±0.17 and 1.41±0.17 nmol/mg protein, respectively, p <0.05) (**Table 3.5A** and **Figure 3.9**). Glutathione concentrations were slightly decreased in livers of the HF-fed rats following intervention with chitosan (Δ = -0.32±0.07 nmol/mg protein). However, the chitosan did not affect levels of liver glutathione concentrations of the NF-fed diets (Δ = -0.32±0.07 nmol/mg protein). The results imply that these two compounds may interfere or inhibit absorption of some nutrients essential for de novo synthesis of liver glutathione.

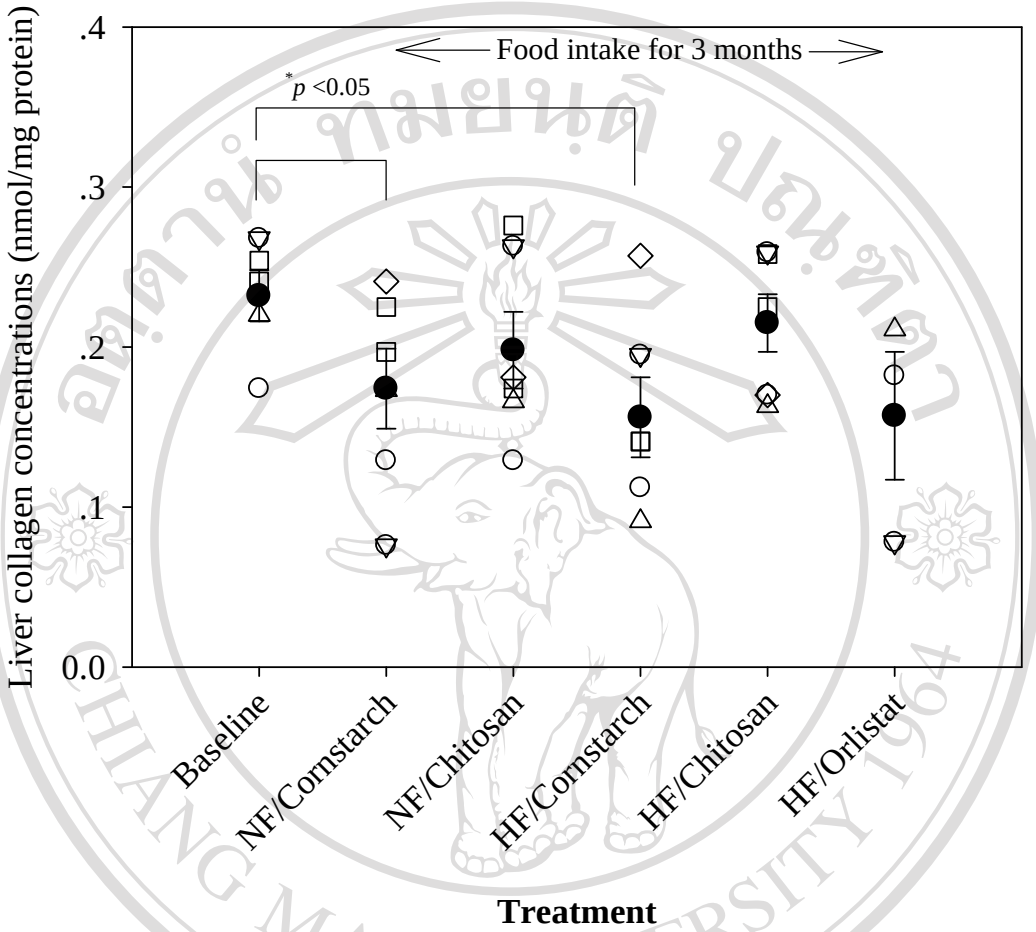


Figure 3-9 Liver collagen concentrations of NF- and HF-fed rats treated with cornstarch, chitosan and orlistat. Data are expressed as individual and mean±SD values. $^{*}p$ <0.05 when compared to baseline.

While growing up, rats' liver collagen concentrations were decreased significantly (from 0.232±0.016 nmol/mg protein to 0.174±0.025 nmol/mg protein after feeding with NF diet, and to 0.156±0.025 nmol/mg protein after feeding with HF diet) (**Table 3.5A** and **Figure 3.9**). A hypolipidemic drug orlistat did not change any levels of the liver collagen concentrations. Chitosan slightly increased the glutathione concentration in livers of the NF-fed rats, and replete liver glutathione concentrations up to the baseline levels (0.215±0.018 nmol/mg protein) of the HF-fed rats.

Table 3.7B Levels of liver glutathione (GSH) and collagen of wild type (WT) mice fed with Fe diets together with oral administration (gavaging) of PBS, GTE and DFP. Data are expressed as individual and mean±SD values.

Mice	Treatment	No.	GSH content (nmol/mg protein)	Collagen content (nmol/mg protein)
WT	Normal diet	1	6.00	0.039
		2	2.45	0.019
		3	0.43	0.017
		4	4.93	0.018
		Mean±SEM	3.45±1.25	0.023±0.005
WT	Fe diet/Placebo	1	3.41	0.042
		2	3.42	0.039
		3	5.00	0.034
		4	3.96	0.051
		Mean±SEM	3.95±0.37	0.041±0.004
WT	Fe diet/GTE	1	6.95	0.035
		2	3.58	0.018
		3	0.10	0.033
		4	5.55	0.060
		Mean±SEM	4.04±1.48	0.037±0.009
WT	Fe diet/DFP	1	4.81	0.025
		2	3.68	0.018
		3	2.88	0.025
		4	5.76	0.089
		Mean±SEM	4.28±0.63	0.039±0.017

Table 3.7C Levels of liver glutathione (GSH) and collagen of heterozygous β-knockout (BKO) mice fed with Fe diets together with oral administration (gavaging) of PBS, GTE and DFP. Data are expressed as individual and mean±SD values.

Mice	Treatment	No.	GSH content (nmol/mg protein)	collagen content (nmol/mg protein)
BKO	Fe diet/Placebo	1	5.11	0.018
		2	3.53	0.022
		Mean±SEM	4.32±0.79	0.020±0.002
BKO	Fe diet/GTE	1	3.99	0.028
		2	4.29	0.020
		3	2.31	0.028
		Mean±SEM	3.53±0.62	0.025±0.003
BKO	Fe diet/DFP	1	2.50	0.030
		2	3.71	0.014
		Mean±SEM	3.10±0.60	0.022±0.008

Study in mice

Similarly, diet increased levels of liver glutathione and collagen concentrations of WT mice (**Table 3.5B**, **Figure 3.11** and **Figure 3.12**). GTE and DFP did not much change any levels of liver glutathione and collagen in WT mice, and levels of liver collagen in BKO thalassemic mice. Unexpectedly, the GTE and DFP decreased levels of liver glutathione in BKO thalassemic mice slightly and insignificantly (from 4.32±0.79 nmol/mg protein to 3.53±0.62 and 3.10±0.60 nmol/mg protein, respectively) (**Table 3.5B**, **Table 3.5C** and **Figure 3.11**).

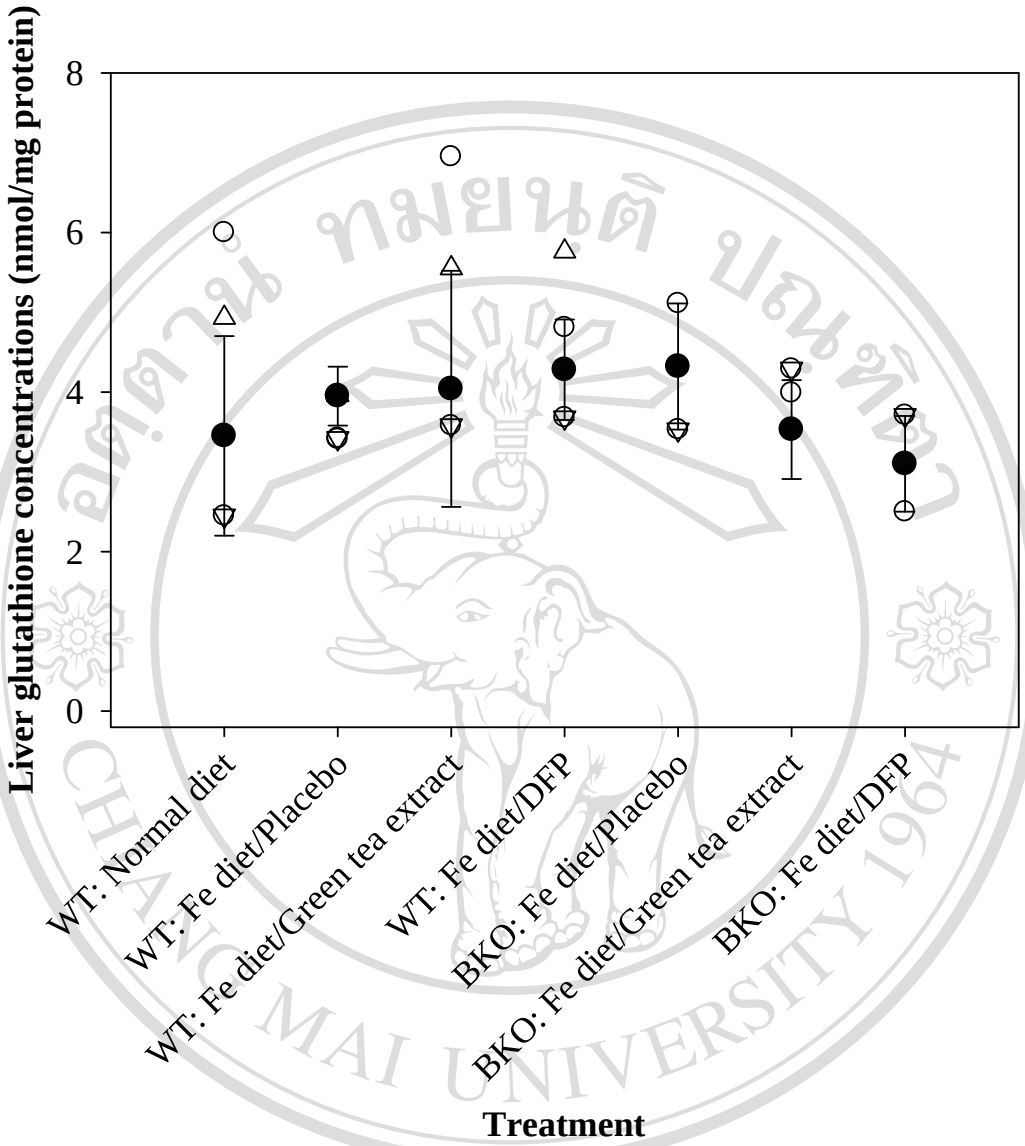


Figure 3-10 Levels of liver reduced glutathione (GSH) of the wild type (WT) and heterozygous β-knockout (BKO) mice fed with Fe diets together with oral administration (gavaging) of PBS (placebo), GTE and DFP for 2 months. Data are expressed as individual and mean±SD values.

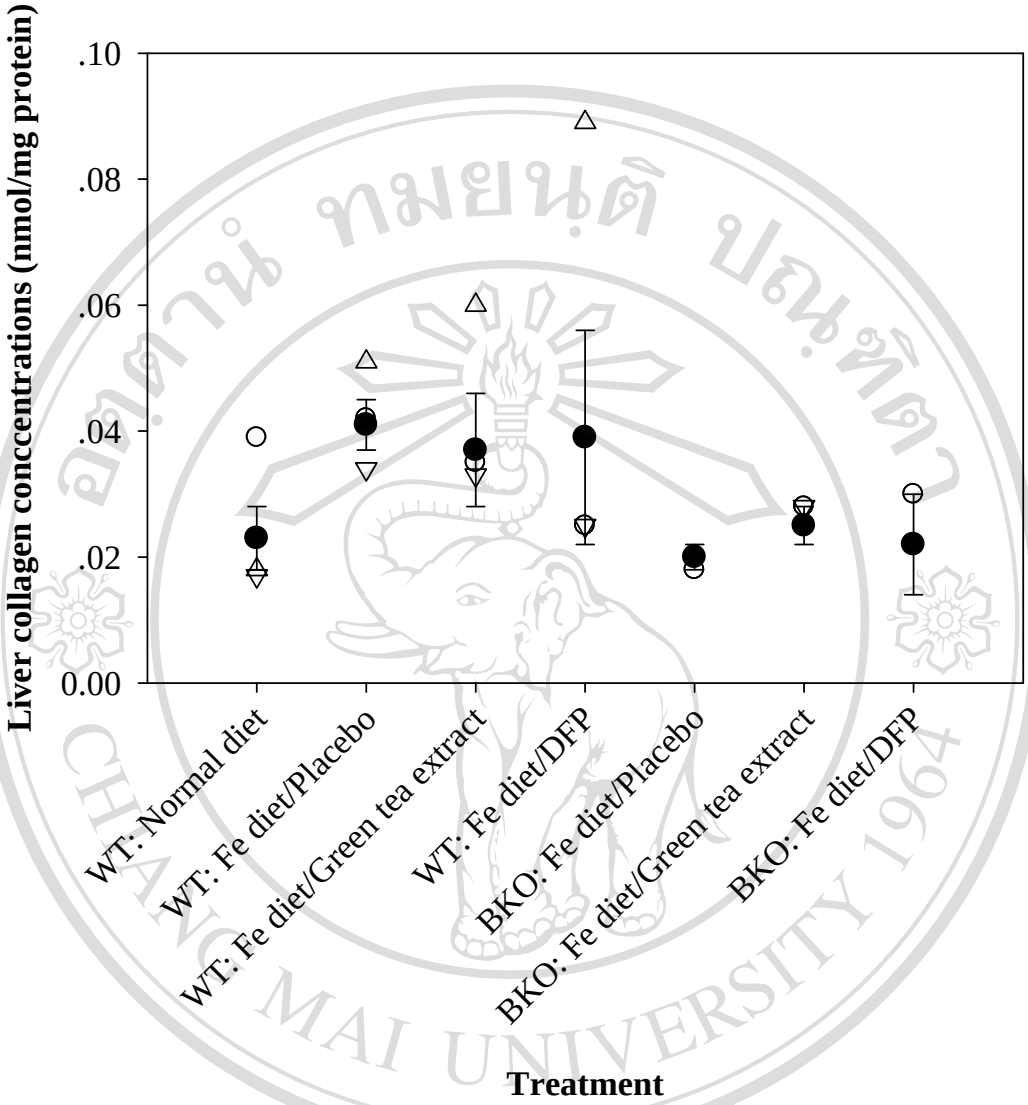


Figure 3-11 Levels of liver collagen of the wild type (WT) and heterozygous β-knockout (BKO) mice fed with Fe diets together with oral administration (gavaging) of PBS (placebo), GTE and DFP for 2 months. Data are expressed as individual and mean±SD values.

3.5 Effects of chitosan on liver malondialdehyde concentrations

In **Table 3.6A** and **Figure 3.13**, the rats which were fed with HF diet had higher liver malondialdehyde (MDA) concentrations (28.71±2.90 nmol/mg protein) than those fed with NF diet (22.28±2.16 nmol/mg protein). Most importantly, chitosan was able to abate a progressive increase of the liver MDA concentrations in the NF-fed rats (from 22.28±2.16 to 17.66±2.12 nmol/mg protein) and in HF-fed rats (from 28.71±2.90 to 21.95±3.46 nmol/mg protein) as well. Orlistat was the most effective in decreasing the liver MDA concentrations of the HF-fed rats (p <0.05).

Table 3-8A Levels of liver malondialdehyde (MDA) of NF- and HF-fed rats treated with cornstarch, chitosan and orlistat. Data are expressed as individual and mean±SD values. $^{*}p$ <0.05 when compared to baseline, $^{\#}p$ <0.05 when compared to cornstarch.

Treatment	No.	NF diet	HF diet
		MDA concentration (nmol/mg protein)	MDA concentration (nmol/mg protein)
Baseline	1	21.62	21.62
	2	16.62	16.62
	3	12.12	12.12
	4	9.31	9.31
	5	11.86	11.86
	Mean±SEM	14.31±2.17	14.31±2.17
Cornstarch	1	24.46	18.93
	2	21.48	36.97
	3	25.83	30.10
	4	11.99	27.54
	5	26.06	30.01
	6	23.88	ND
	Mean±SEM	22.28±2.16*	28.71±2.90*
Chitosan	1	17.91	16.81
	2	27.49	16.36
	3	13.82	33.35
	4	14.51	12.87
	5	13.95	20.99
	6	18.31	31.35
	Mean±SEM	17.66±2.12	21.95±3.46
Orlistat	1	-	19.38
	2	-	13.25
	3	-	21.42
	Mean±SEM	-	18.02±2.45$^{\#}$

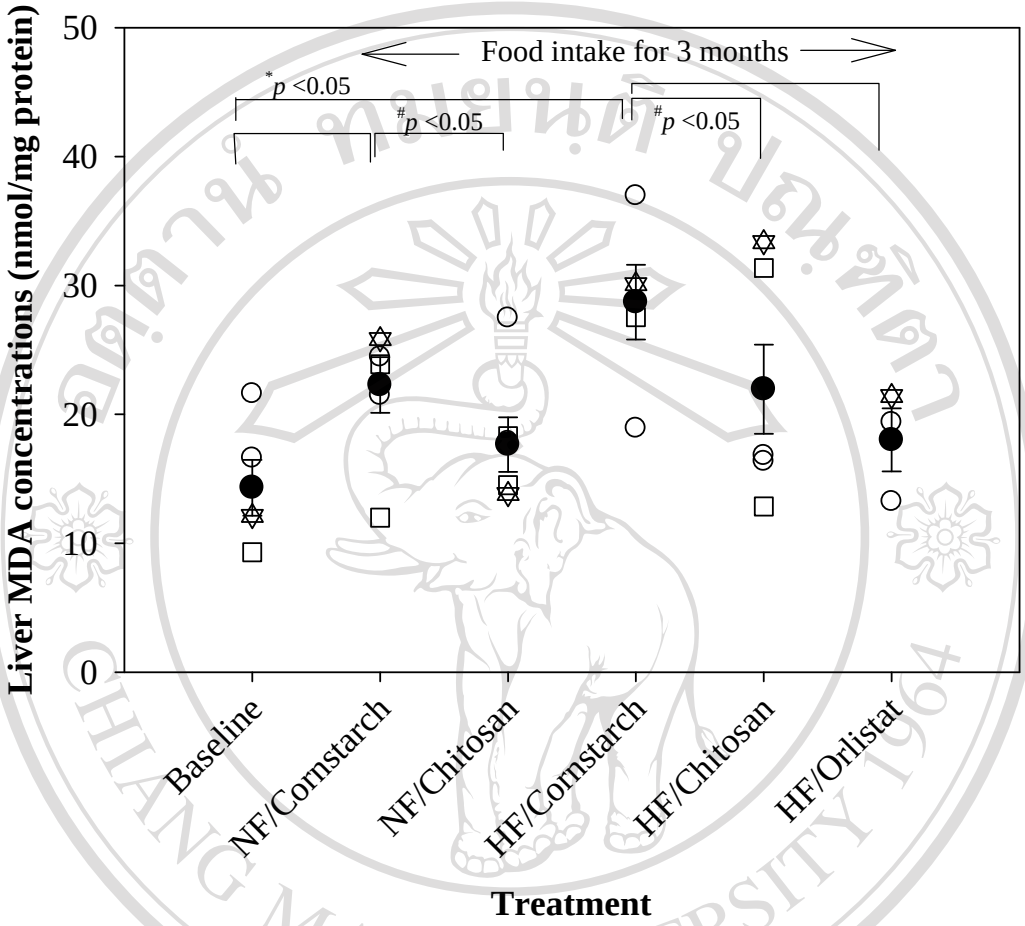


Figure 3-12 Liver malondialdehyde (MDA) of the NF diet- and HF diet-fed rats which were treated with cornstarch, chitosan and orlistat for 3 months. Data are expressed as individual and mean±SD values. $^{*}p$ <0.05 when compared to baseline, $^{\#}p$ <0.05 when compared to cornstarch.

3.6 Effects of green tea extract on liver malondialdehyde concentrations

Administration of dietary iron (ferrocene-supplemented diet or Fe diet) markedly increased liver MDA concentrations of the WT mice (**Table 3.6B** and **Figure 3.14**). DFP was able to lower the liver MDA concentrations, most importantly

Table 3-8B Levels of liver malondialdehyde (MDA) of wild type (WT) mice fed with Fe diets together with oral administration of PBS, GTE and DFP. Data are expressed as individual and mean±SD values. $^{*}p$ <0.05 when compared to Fe diet/Placebo.

Mice	Treatment	No.	MDA concentration	
			nmol/mg protein	nmol/ mg dry weight
WT	Normal diet	1	1.30	0.025
		2	0.58	0.011
		3	1.51	0.029
		4	5.98	0.100
		Mean±SEM	2.34±1.23	0.041±0.020
WT	Fe diet/Placebo	1	2.25	0.051
		2	4.20	0.099
		3	4.20	0.083
		4	1.67	0.031
		Mean±SEM	3.08±0.66	0.066±0.015
WT	Fe diet/GTE	1	0.63	0.011
		2	0.64	0.011
		3	0.07	0.001
		4	0.07	0.001
		Mean±SEM	0.35±0.16*	0.006±0.003*
WT	Fe diet/DFP	1	4.30	0.079
		2	0.30	0.006
		3	1.38	0.025
		4	0.44	0.009
		Mean±SEM	1.61±0.93	0.030±0.017

GTE was effective in decreasing approximately ten-fold concentrations of the liver MDA (p <0.05). The results suggest that GTE which contains polyphenolic catechins, particularly EGCG would do bifunctional activities, iron-chelating and free-radicals scavenging properties, that can inhibit ROS-induced lipid peroxidation in the iron0loaded hepatocytes. It is observed that treatment of GTE and DFP for 2 months did not affect levels of the liver MDA concentrations (0.47±0.29 and 0.46±0.07 nmol/mg protein, respectively) when compared to untreatment (0.38±0.09 nmol/mg protein) (**Table 3.6C**, **Figure 3.14** and **Figure 3.15**).

Table 3-8C Levels of liver malondialdehyde (MDA) of heterozygous β-knockout (BKO) mice fed with Fe diets together with oral administration of PBS, GTE and DFP. Data are expressed as individual and mean±SD values.

Mice	**Treatment**	**No.**	**MDA concentration**	
			nmol/mg protein	nmol/mg dry weight
BKO	Fe diet/Placebo	1	0.29	0.006
		2	0.47	0.010
		Mean±SEM	0.38±0.09	0.008±0.002
BKO	Fe diet/GTE	1	0.09	0.002
		2	1.33	0.032
		3	0.12	0.003
		4	0.36	0.007
		Mean±SEM	0.47±0.29	0.011±0.007
BKO	Fe diet/DFP	1	0.53	0.010
		2	0.39	0.008
		Mean±SEM	0.46±0.07	0.009±0.001

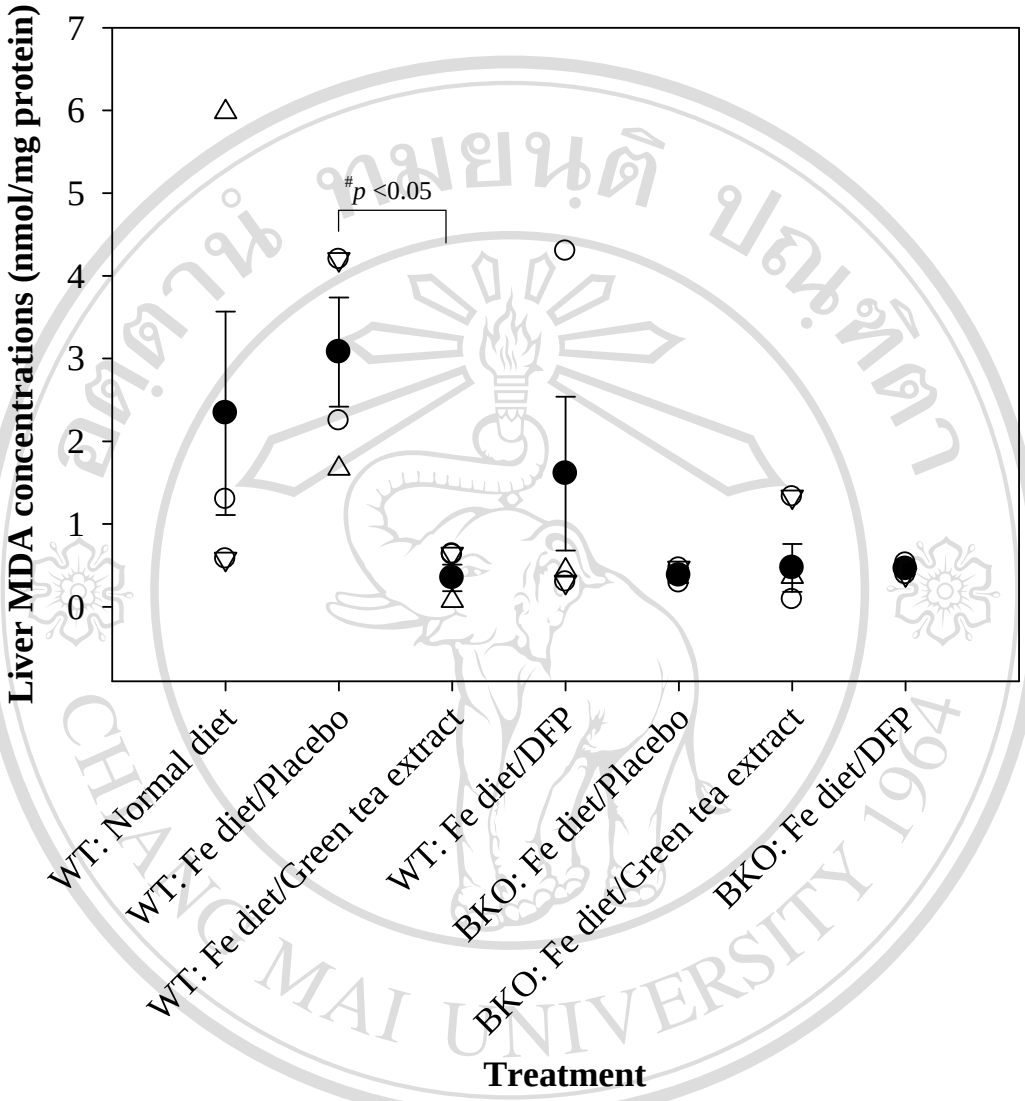


Figure 3-13 Levels of liver malondialdehyde (MDA) concentration of the wild type (WT) and heterozygous β-knockout (BKO) mice fed with Fe diets together with oral administration (gavaging) of PBS (placebo), GTE and DFP for 2 months. Data are expressed as individual and mean±SD values. $^{\#}p$ <0.05 when compared to Fe diet/Placebo.

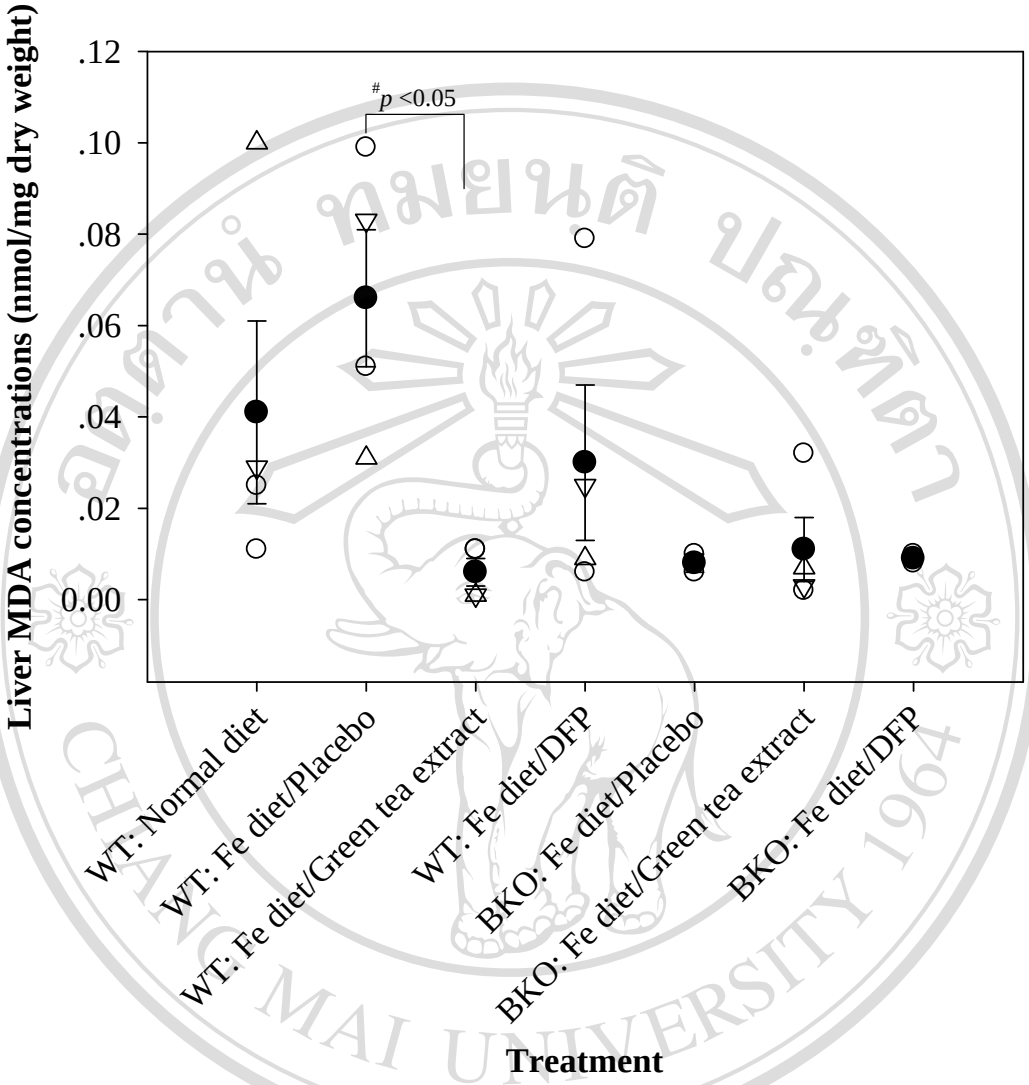


Figure 3-14 Levels of liver malondialdehyde (MDA) concentration of the wild type (WT) and heterozygous β-knockout (BKO) mice fed with Fe diets together with oral administration (gavaging) of PBS (placebo), GTE and DFP for 2 months. Data are expressed as individual and mean±SD values. $^{\#}p$ <0.05 when compared to Fe diet/Placebo.

3.7 Effects of high-fat diet and chitosan on liver functions

Measurement of liver enzyme activity

Results in **Table 3.7** and **Figure 3.16** showed that HF diet contributed to the increase of plasma AST activity (p <0.05), but not to the plasma ALT activity.

Table 3-9 Levels of plasma ALT and AST of NF- and HF-fed rats treated with cornstarch, chitosan and orlistat. Data (U/L) are expressed as individual and mean±SD values.

Treatment	No.	NF diet		HF diet	
		ALT	AST	ALT	AST
Baseline	1	29	30	29	30
	2	27	29	27	29
	3	32	28	32	28
	4	28	30	28	30
	5	29	32	29	32
	Mean±SEM	29±2	30±1	29±2	30±1
Cornstarch	1	29	30	29	29
	2	32	31	29	31
	3	31	32	28	30
	4	29	31	31	33
	5	32	33	32	33
	6	33	33	31	32
	Mean±SEM	31±2	32±1*	30±2	31±2*
Chitosan	1	32	33	33	34
	2	31	30	29	30
	3	28	31	31	32
	4	30	31	32	33
	5	32	33	32	30
	6	31	32	31	32
	Mean±SEM	31±2	32±1	33±2	34±2
Orlistat	1	-	-	30	32
	2	-	-	32	34
	3	-	-	30	32
	Mean±SEM	-	-	31±1#	33±1#

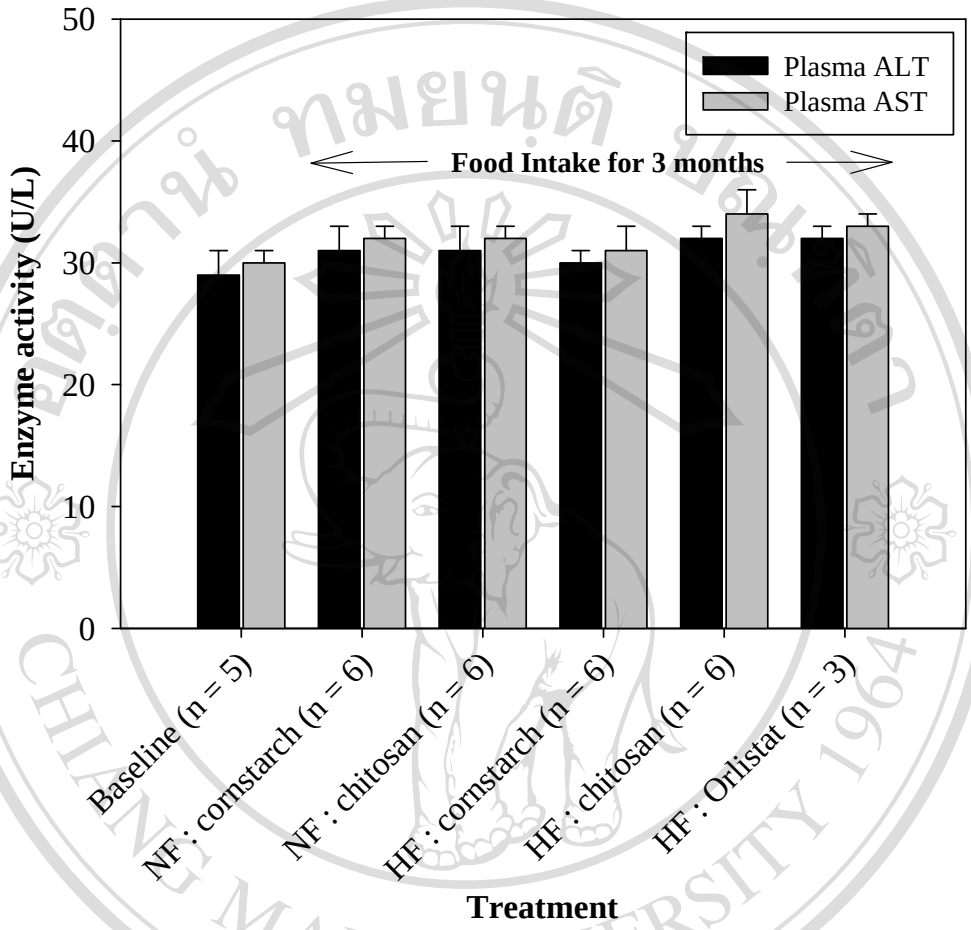


Figure 3-15 Levels of plasma ALT and AST activities of the NF diet- and HF diet-fed rats which were treated with cornstarch, chitosan and orlistat for 3 months. Data are expressed as individual and mean±SD values.

However, orlistat slightly increased levels of plasma AST and ALT activities in both NF-fed and HF-fed diet, suggesting it has not side effects on their liver functions. Chitosan slightly increased levels of the plasma ALT activity; nonetheless, the increased ALT levels were not so high that it indicated liver toxicity. The data point out that the chitosan treatment might affect functions of the rats' livers mildly, but not seriously.

Histochemical examinations

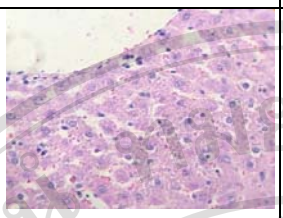
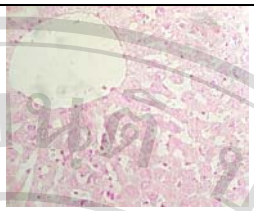
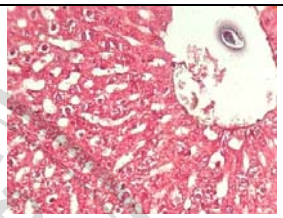
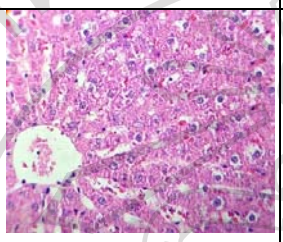
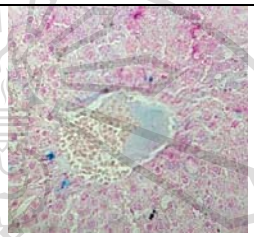
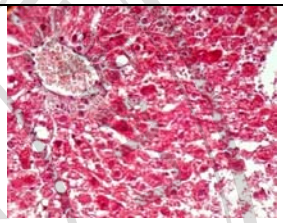
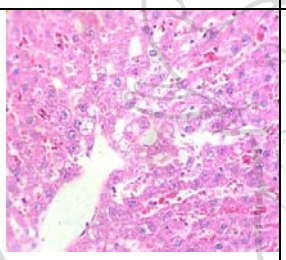
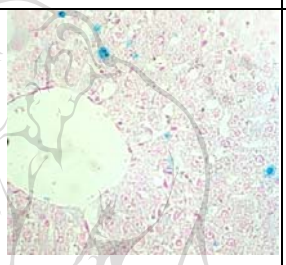
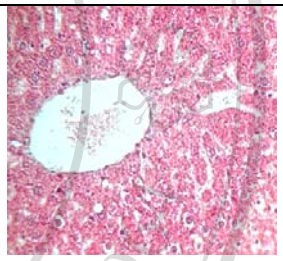
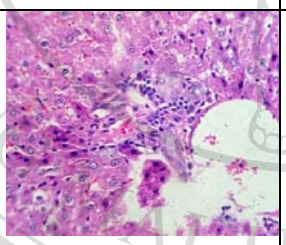
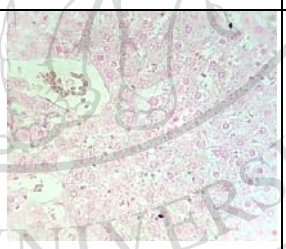
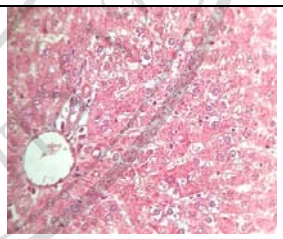
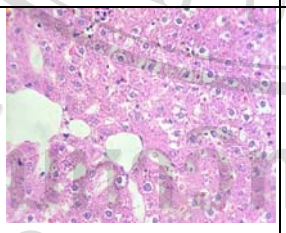
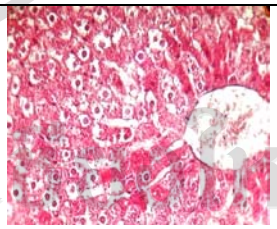
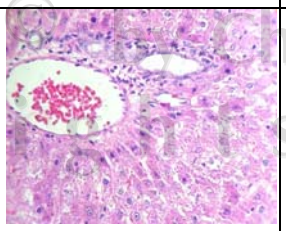
Treatment	H & E stain (x 40)	Pearl's stain	Mason trichrome
Normal (Code 4/1)			
NF diet/ Cornstarch (Code 8/5)			
NF diet/ Chitosan (Code 30/5)			
HF diet/ Cornstarch (Code 2/5)			
HF diet/ Chitosan (Code 17/3)			
HF diet/ Orlistat (Code 23/5)		ND	ND

Figure 3-16 Histochemical examinations of the NF diet- and HF diet-fed rats which were treated with cornstarch, chitosan and orlistat for 3 months.

In H&E staining results, the tissue slide (Code 4/1) obtained from liver of a normal rat (control) showed no significant pathologic change. The tissue slide (Code 8/5) obtained from liver of a NF-fed rat treated with cornstarch showed fatty metamorphosis, centrilobular, mild degree, macrovesicular and microvesicular pattern**.** Increased collagen deposit was not demonstrated. The tissue slide (Code 30/5) obtained from liver of a NF-fed rat treated with chitosan showed portal inflammation, mild by eosinophils and lymphocytes**.** No increased collagen deposit is demonstrated**.** The tissue slide (Code 2/5) obtained from liver of a HF-fed rat treated chitosan showed fatty metamorphosis, centrilobular, mild degree, macrovesicular pattern**.** Increased collagen deposit was not demonstrated**.** The tissue slide (Code 17/3) obtained from liver of a HF-fed rat reated with chitosan showed rare aggregates of granulocytes, neutrophils and eosinophils, in hepatic sinusoids**.** No significant pathologic change was found. The tissue slide (Code 23/5) obtained from liver of a HF-fed rat treated with orlistat showed portal inflammation, moderate degree by lymphocytes**.** No increased collagen deposit is demonstrated. **(Figure 3-16).**

Pearl's staining results observed in all slides of rats' liver tissues did not show any abnormal accumulation of iron (deep blue-colored granules), demonstrating that dietary fat did not enhance increase of iron deposition in liver cells.

In **Fiure 3-17** Pearl's staining observation indicates that Fe diet enhanced iron deposition in the hepatocytes of both WT and BKO thalassemic mice. Incredibly, GT was efficient in reducing the liver iron accumulation.

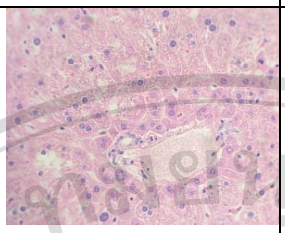
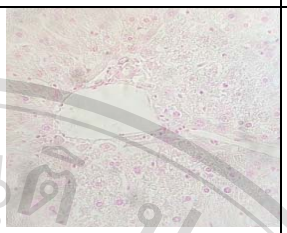
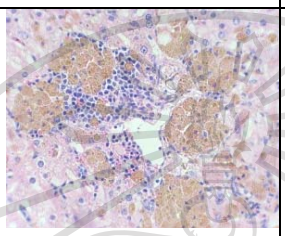
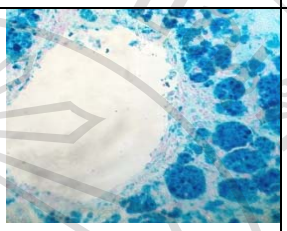
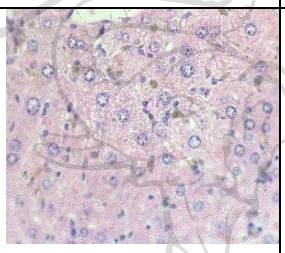
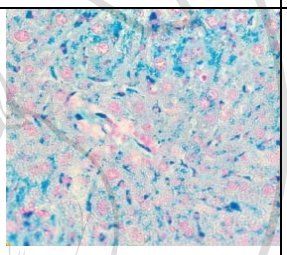
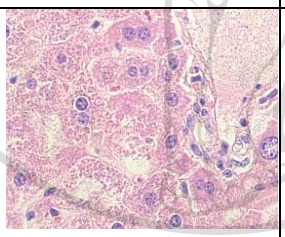
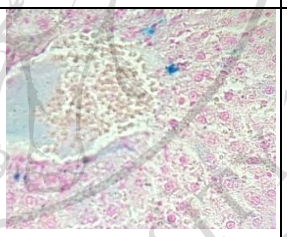
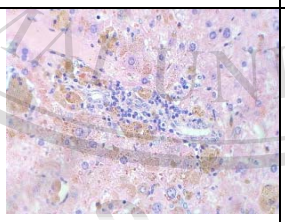
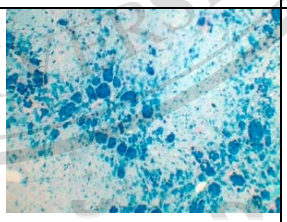
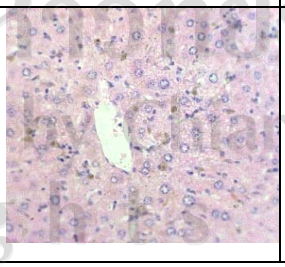
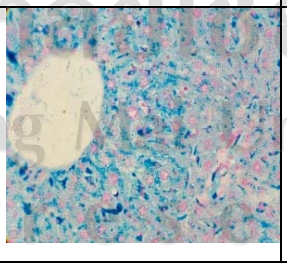
Mice	Treatment	H&E stain (x 40)	Pearl's stain	Mason trichrome
WT	Normal diet			ND
WT	Fe diet			ND
WT	Fe diet/GTE			ND
BKO	Normal diet			ND
BKO	Fe diet			ND
BKO	Fe diet/GTE			ND

Figure 3-17 Histochemical examinations of the wild type (WT) and heterozygous β-knockout (BKO) mice fed with Fe diets together with oral administration (gavaging) of PBS (placebo), GTE and DFP for 2 months. ND = Not done.