CHAPTER III

RESULTS

Animal characteristics

Initial body weights for each of the experimental groups were in the range of 135-160 g and there were no different among the experimental groups (140.71 ± 8.20, 139.40 ± 6.78, 140.63 ± 7.99, 156.10 ± 3.94, 140.44 ± 8.24, 139.58 ± 4.94, 140.00 ± 5.71 and 153.91 ± 5.58 g in NDC, ND_{30}, ND_{60}, ND_{90}, HDC, HD_{30}, HD_{60} and HD_{90} groups, respectively). As presented in Table 4, the body weight was 440.71±10.66 g and weight gain was about 220% in NDC group after 12 weeks of dietary period. Curcuminoids administration for 12 weeks had no effects on body weight and visceral fat in rats fed normal diets. Weight gains in ND_{30}, ND_{60}, and ND_{90} groups (226, 208 and 189%, respectively) were nearly equal to that in NDC group and the body weights were 441.60±19.37, 420.13±7.04, and 446.60±8.09 g, respectively. Visceral fat masses in NDC, ND_{30}, ND_{60}, and ND_{90} groups were 26.37±2.11, 25.34±2.06, 24.15±2.20 and 25.11±1.63 g, respectively. High-fat diet administration resulted in an increase of body weight, accompanied by accumulation of visceral fat. At week 12, weight gain was about 272% in HDC group. The body weight and visceral fat mass were significantly greater in HDC group (510.00±18.28 and 47.30±3.89 g, respectively) (both $p<0.05$) than in NDC group. Weight gains in HD_{30}, HD_{60}, and HD_{90} groups were 266, 274 and 232%, respectively. In addition, the body weights and visceral fat masses in HD_{30}, HD_{60}, and HD_{90} groups (502.92±17.76 and 46.34±3.52, 517.67±16.59 and 46.52±3.44, and 503.82±10.91 and 39.98±2.09 g,

respectively) were comparable to HDC group. An analysis of dietary records showed that average daily energy intake over 12 weeks of dietary period were also not significantly different between NDC, ND_{30}, ND_{60}, and ND_{90} groups (61.63±1.93, 61.67± 2.73, 59.80±1.29, 64.62±1.04 kcal/day, respectively). There was significantly higher average daily energy intake in HDC group (102.99±3.51 kcal/day, $p<0.05$) than in NDC group. Compared with HDC group, the average daily energy intakes in HD_{30}, HD_{60} and HD_{90} groups were not significantly different (108.89±3.06, 107.32±3.73 and 97.85±3.10, respectively, $p>0.05$). However, the average daily food intakes in HD_{90} group was apparently lower than HD_{30} and HD_{60} groups (both $p<0.05$).

Table 4 Effects of curcuminoids administration on body weight, energy intake, and visceral fat in high-fat diet induced obese rats.

	NDC	**ND_{30}**	**ND_{60}**	**ND_{90}**	**HDC**	**HD_{30}**	**HD_{60}**	**HD_{60}**
Body weight, g	440.71±10.66	441.60±19.37	420.13±7.04	446.60±8.09	510.00±18.28 [a]	502.92±17.76	517.67±16.59	503.82±10.91
Visceral fat, g	26.37±2.11	25.34±2.06	24.15±2.20	25.11±1.63	47.30±3.89 [a]	46.34±3.52	46.52±3.44	39.97±2.09
Energy intake, kcal/day	61.63±1.93	61.67±2.73	59.80±1.29	64.62±1.04	102.99±3.51 [a]	108.89±3.06	107.32±3.73	97.85±3.10 [f,g]

Each value is mean ± SE from 7-12 rats. NDC; normal diet control, ND_{30}; normal diet + curcuminoids administration at dose of 30 mg/kg BW, ND_{60}; normal diet + curcuminoids administration at dose of 60 mg/kg BW, ND_{90}; normal diet + curcuminoids administration at dose of 90 mg/kg BW, HDC; high-fat diet control, HD_{30}; high-fat diet + curcuminoids administration at dose of 30 mg/kg BW, HD_{60}; high-fat diet + curcuminoids administration at dose of 60 mg/kg BW, HD_{90}; high-fat diet + curcuminoids administration at dose of 90 mg/kg BW. [a]; significantly different from NDC group, [f]; significantly different from HD_{30} group, [g]; significantly different from HD_{60} group, $p<0.05$.

Fasting plasma parameters

The effect of curcuminoids treatment on plasma parameters in high-fat induced obese rat is summarized in Table 5. Fasting plasma glucose levels in rats received normal diets with and without curcuminoids after 12 weeks of dietary period were not different (132.01±4.62, 136.05±7.48, 122.91±6.46, and 128.30±4.52 mg/dl in NDC, ND_{30}, ND_{60}, and ND_{90} groups, respectively). There were also no differences in fasting plasma insulin concentrations at week 12 among the NDC, ND_{30}, ND_{60}, and ND_{90} groups (1.24±0.13, 1.19±0.31, 1.34±0.23 and 1.40±0.10 ng/ml, respectively). High-fat feeding for 12 weeks resulted in a significantly elevated fasting plasma glucose levels (153.54±4.81 mg/dl, $p<0.05$). Compared with NDC group, however, the fasting plasma insulin levels in HDC group was not significantly different (1.35±0.11 ng/ml, $p>0.05$). Curcuminoids administration at all doses (30, 60, and 90 mg/kg BW) significantly decreased fasting plasma glucose concentrations in rats fed high-fat diets (132.04±5.60, 134.69±7.12 and 136.18±4.08 mg/dl in HD_{30}, HD_{60}, and HD_{90} groups, respectively, $p<0.05$) whereas it had no effect on fasting plasma insulin levels (1.56±0.61, 1.51±0.21 and 1.40±0.25 ng/ml in HD_{30}, HD_{60} and HD_{90} groups, respectively). The degree of insulin resistance was determined using HOMA index. The HOMA indexes were 11.13±1.35, 10.89±2.45, 10.44±1.81 and 11.38±0.96 in NDC, ND_{30}, ND_{60}, and ND_{90} groups, respectively). Although the HOMA index tended to be higher in HDC group (13.67±1.57) than in NDC group, there was no significant difference. Compared with HDC group, the HOMA indexes in HD_{30}, HD_{60}, HD_{90} groups were also no significant difference (14.41±2.34, 14.23±1.67 and 11.97±2.23, respectively).

The range of fasting plasma triglyceride levels at week 12 in rats fed normal diet with or without curcuminoids administration was 62-97 mg/dl (85.14±20.31, 97.24±7.69, 82.24±6.71 and 62.77±15.64 mg/dl in NDC, ND_{30}, ND_{60}, and ND_{90} groups, respectively). High-fat feeding led to an increase of plasma triglyceride levels (110.27±17.37 mg/dl) although it was not significantly different from the value obtained from NDC group *($p>0.05$*). The fasting plasma triglyceride levels in HD_{30}, HD_{60} groups (109.51±8.97 and 93.76±9.29 mg/dl) were comparable to HDC group. In contrast, high-fat fed rats treated with curcuminoids at dose of 90 mg/kg BW had a significant decrease of plasma triglyceride concentrations (56.10±4.42 mg/dl in HD_{90} group) compared with HDC, HD_{30}, HD_{60} groups (all $p<0.05$).

Fasting plasma free fatty acid levels at week 12 were similar among NDC, ND_{30}, ND_{60}, and ND_{90} groups (0.38±0.05, 0.34±0.04, 0.35±0.02 and 0.36±0.05, respectively). Compared with NDC group, the fasting plasma free fatty acid concentrations was significantly elevated in HDC group (0.51± 0.04 mmol/l, $p<0.05$). There were significant decreases of fasting plasma free fatty acid levels in high-fat fed rats treated with curcuminoids at doses of 30, 60, and 90 mg/kg BW (0.38±0.03, 0.36±0.03 and 0.32±0.01 mmol/l, respectively, all $p<0.05$).

Figure 3 shows the association between plasma free fatty acid levels and obesity. The data from both the NDC and HDC groups were combined. There was no correlation between plasma free fatty acid level and body weight ($p>0.05$). By contrast, the elevated plasma free fatty acid level was significantly correlated with an increase of visceral fat mass ($r = 0.546$, $p<0.05$).

Table 5 Effects of curcuminoids administration on fasting plasma parameters and HOMA index in high-fat diet induced obese rats.

	NDC	**ND_{30}**	**ND_{60}**	**ND_{90}**	**HDC**	**HD_{30}**	**HD_{60}**	**HD_{90}**
Glucose, mg/dl	132.01±4.62	136.05±7.48	122.91±6.46	128.30±4.52	153.54±4.81 [a]	132.04±5.60 [e]	134.69±7.12 [e]	136.18±4.08 [e]
Insulin, ng/ml	1.24±0.13	1.19±0.31	1.34±0.23	1.40±0.10	1.35±0.11	1.56±0.61	1.51±0.21	1.40±0.25
FFA, mmol/l	0.38±0.05	0.34±0.04	0.35±0.02	0.36±0.05	0.51±0.04 [a]	0.38±0.03 [e]	0.36±0.03 [e]	0.32±0.01 [e]
TG, mg/dl	85.14±20.31	97.24±7.69	82.24±6.71	62.77±15.64	110.27±17.37	109.51±8.97	93.76±9.29	56.10±4.42 [e,f,g]
HOMA index	11.13±1.35	10.89±2.45	10.44±1.81	11.38±0.96	13.67±1.57	14.41±2.34	14.23±1.67	11.97±2.23

FFA; free fatty acid, TG; triglyceride. Each value is mean ± SE from 7 -12 rats. NDC; normal diet control, ND_{30}; normal diet + curcuminoids administration at dose of 30 mg/kg BW, ND_{60}; normal diet + curcuminoids administration at dose of 60 mg/kg BW, ND_{90}; normal diet + curcuminoids administration at dose of 90 mg/kg BW, HDC; high-fat diet control, HD_{30}; high-fat diet + curcuminoids administration at dose of 30 mg/kg BW, HD_{60}; high-fat diet + curcuminoids administration at dose of 60 mg/kg BW, HD_{90}; high-fat diet + curcuminoids administration at dose of 90 mg/kg BW. [a]; significantly different from NDC group. [e]; significantly different from HDC group, [f]; significantly different from HD_{30} group, [g]; significantly different from HD_{60} group, $p<0.05$.

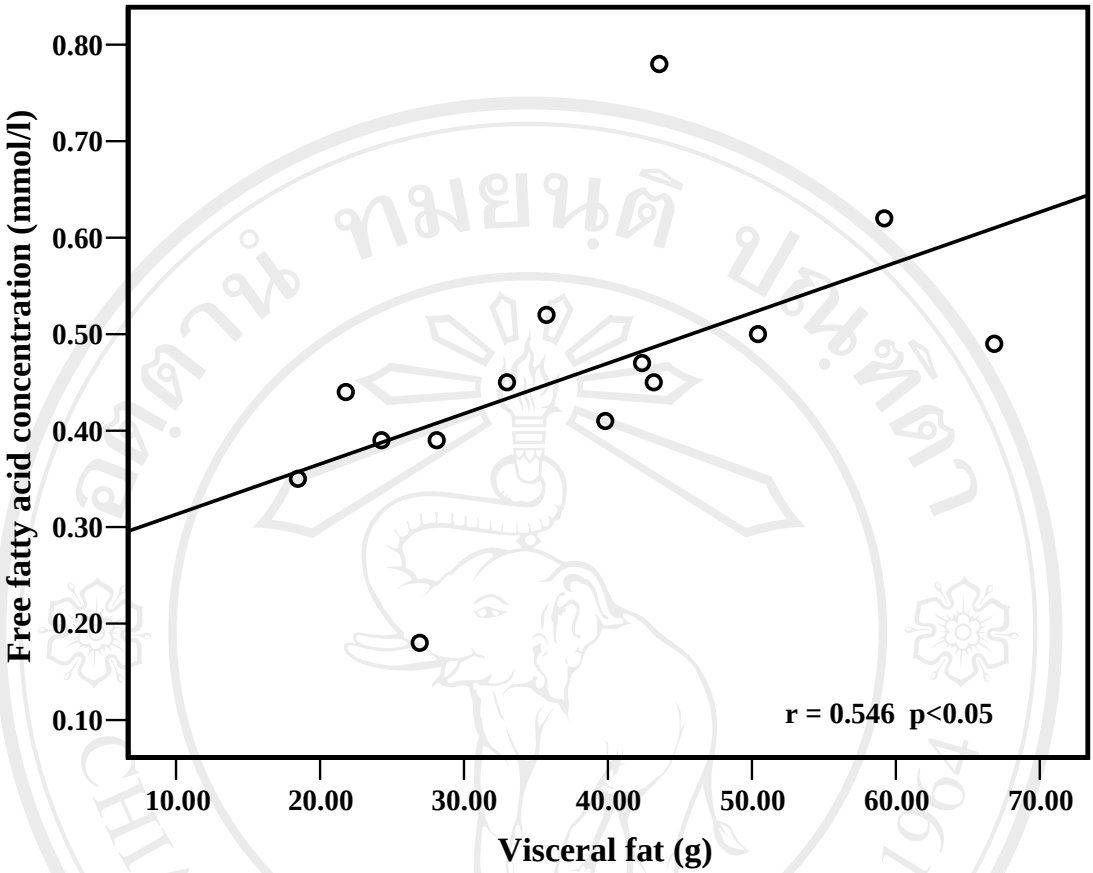

Figure 3 Correlation between visceral fat and plasma free fatty acid concentrations in rats received normal diet or high-fat diet for 12 weeks.

Tissue-triglyceride content

Table 6 presents triglyceride contents in liver, soleus and red gastrocnemius muscles after 12 weeks of diet intervention and curcuminoids administration. There was no difference in hepatic triglyceride contents among rats fed normal diets alone or with curcuminoids administration (10.75±1.02, 10.47±2.50, 8.80±0.95 and 11.23±2.24 mg/gm tissues in NDC, ND_{30}, ND_{60}, and ND_{90} groups, respectively). High-fat feeding resulted in a significant elevation of hepatic triglyceride levels (36.06±4.99 mg/gm tissues, $p<0.05$). Curcuminoids administration at all doses (30, 60 and 90 mg/kg BW) tended to reduced the increased hepatic triglyceride levels associated with high-fat feeding (25.01±1.80, 29.63±3.31 and 28.35±4.04 mg/gm tissues in HD_{30}, HD_{60}, HD_{90} groups, respectively) and there was a significant difference in the hepatic triglyceride contents between HDC and HD_{30} groups ($p<0.05$).

The triglyceride contents in muscles, both soleus and red gastrocnemius muscles, were 9.60±2.02 and 5.65±1.69; 8.57±0.81 and 4.40±0.62; 4.99±0.54 and 5.37±0.79; and 11.02±0.85 and 4.80±0.53 mg/gm tissues in NDC, ND_{30}, ND_{60}, and ND_{90} groups, respectively. There was significant difference in soleus triglyceride contents between ND_{60} and ND_{90} groups ($p<0.05$). Compared with NDC group, the triglyceride contents in soleus and red gastrocnemius muscles were not different in rats consumed high-fat diets (9.06±1.47 or 5.50±0.82 mg/gm tissues in HDC group). In addition, curcuminoids treatment at dose of 30 and 60 mg/kg BW had no effect on the triglyceride stored in both muscles. The triglyceride levels in soleus and red gastrocnemius muscles in HD_{30} and HD_{60} groups (8.58±1.50 and 4.27±0.64; and 9.20±1.40 and 3.89±0.50 mg/gm tissues, respectively) were comparable to that

obtained from HDC group. In HD_{90} group, the soleus triglyceride levels was significant higher (12.91±2.30 mg/gm tissues, $p<0.05$) than in HD_{30} group whereas the red gastrocnemius triglyceride level was significantly decreased (3.12±0.32 mg/gm tissues) compared with HDC group ($p<0.05$).

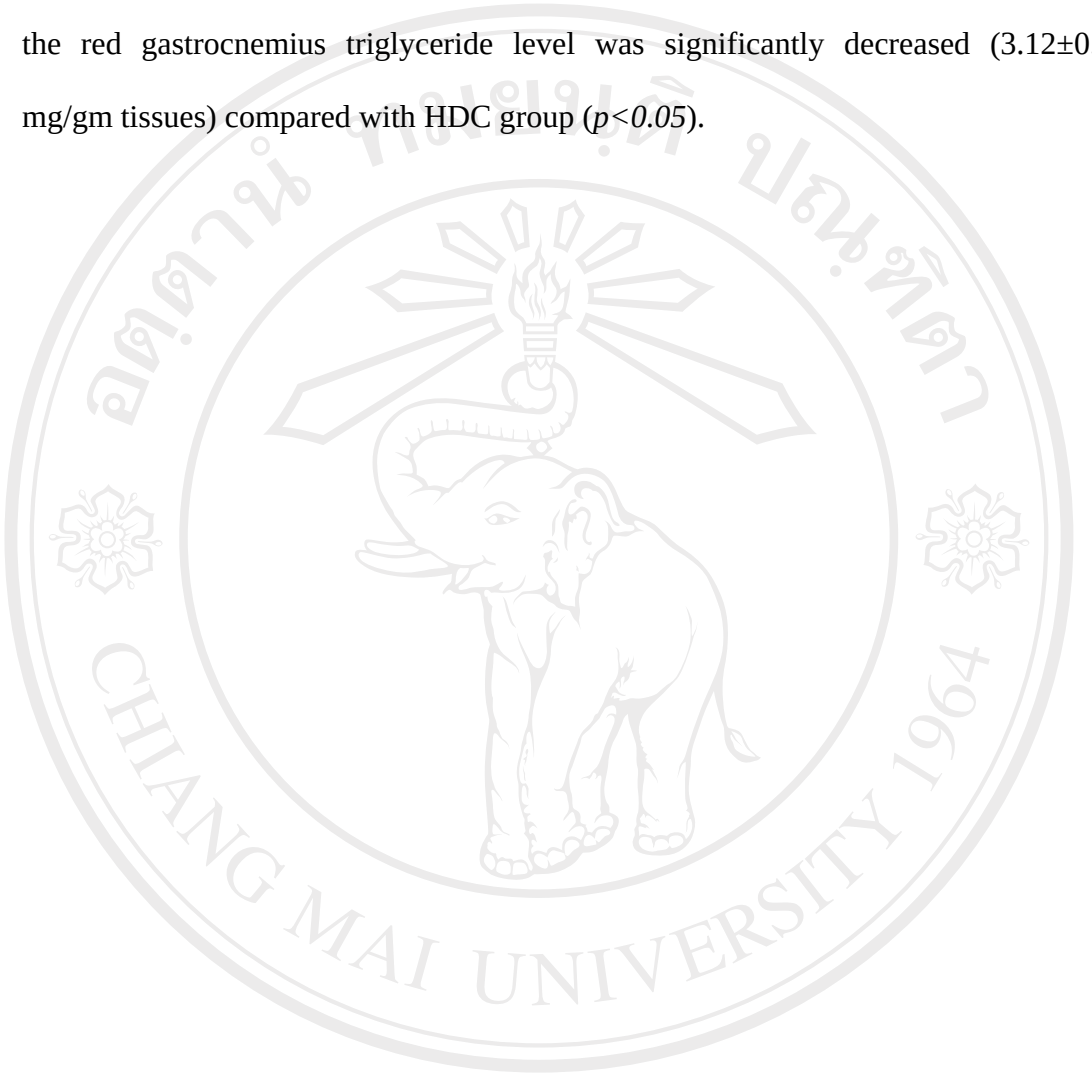

Table 6 Effect of curcuminoids administration on tissues triglyceride concentrations in high-fat diet induced obese rats.

	Triglyceride contents (mg/gm tissue)							
	NDC	**ND_{30}**	**ND_{60}**	**ND_{90}**	**HDC**	**HD_{30}**	**HD_{60}**	**HD_{90}**
Liver	10.75±1.02	10.47±2.50	8.80±0.95	11.23±2.24	36.06±4.99 [a]	25.01±1.80 [e]	29.63±3.31	28.35±4.04
Soleus muscle	9.60±2.02	8.57±0.81	4.99±0.54	11.02±0.85 [c]	9.06±1.47	8.58±1.50	9.20±1.40	12.91±2.30 [f]
Red gastrocnemius muscle	5.65±1.69	4.40±0.62	5.37±0.79	4.80±0.53	5.50±0.82	4.27±0.64	3.89±0.50	3.12±0.32 [e]

Each value is mean ± SE from 7 -12 rats. NDC; normal diet control, ND_{30}; normal diet + curcuminoids at dose of 30 mg/kg BW, ND_{60} ; normal diet + curcuminoids at dose of 60 mg/kg BW, ND_{90} ; normal diet + curcuminoids at dose of 90 mg/kg BW, HDC; high-fat diet control, HD_{30}; high-fat diet + curcuminoids at dose of 30 mg/kg BW, HD_{60} ; high-fat diet + curcuminoids at dose of 60 mg/kg BW, HD_{90} ; high-fat diet + curcuminoids at dose of 90 mg/kg BW. [a] ; significantly different from NDC group, [c] ; significantly different from ND_{60} group, [e] ; significantly different from HDC group, [f] ; significantly different from HD_{30} group, $p<0.05$.

Heart rate variability (HRV)

The cardiac autonomic status was determined by heart rate variability (HRV) measurement. SDNN and RMSSD are represented of time domain parameters. Power spectral parameters calculated are LF, HF and LF/HF ratio. Both LF and HF are expressed in normalized units (LFnu and HFnu). Table 7 presents the baseline data of HRV before the diet intervention. The initial values of SDNN and RMSSD were 2.59±0.42 and 1.82±0.27, 1.22±0.10 and 1.25±0.06, 2.74±0.89 and 1.77±0.29, 2.84±0.54 and 2.24±0.34, 2.77±0.67 and 2.85±0.89, 2.89±0.65 and 2.95±1.10, 2.03±0.43 and 2.60±0.46, 3.77±0.39 and 2.98±0.48 in NDC, ND_{30}, ND_{60}, ND_{90}, HDC, HD_{30}, HD_{60}, and HD_{90} groups, respectively. Also, the baseline values of LFnu, HFnu and LF/HF ration were not different among the experimental groups (0.16±0.00, 0.83±0.01 and 0.19±0.01; 0.17±0.00, 0.83±0.00 and 0.21±0.00; 0.17±0.01, 0.83±0.01 and 0.21±0.01; 0.18±0.01, 0.82±0.01 and 0.22±0.01; 0.16±0.01, 0.84±0.01 and 0.20±0.01; 0.18±0.02, 0.82±0.02 and 0.21±0.03; 0.15±0.01, 0.85±0.01 and 0.17±0.01; and 0.17±0.01, 0.83±0.01 and 0.20±0.01 in NDC, ND_{30}, and ND_{60}, ND_{90}, HDC, HD_{30}, HD_{60}, and HD_{90} groups, respectively).

Table 8 summarizes the results of HRV after 12 weeks of diet intervention and curcuminoids administration. The SDNN and RMSSD at week 12 were comparable among NDC, ND_{30}, and ND_{60} groups (3.41±0.49 and 2.42±0.25; 3.54±0.45 and 2.20±0.22; and 2.73±0.31 and 2.14±0.14, respectively) whereas these values were significantly higher in ND_{90} group (4.37±0.51 and 4.23±0.56, all $p<0.05$). However, curcuminoids administration at all dose (30, 60 and 90 mg/kg BW) had no effects on power spectral parameters. The LFnu, HFnu and LF/HF ratio were comparable among NDC, ND_{30}, ND_{60}, and ND_{90} groups (0.17±0.01, 0.83±0.01 and 0.20±0.01;

0.18±0.00, 0.82±0.00 and 0.21±0.01; 0.18±0.01, 0.82±0.01 and 0.22±0.01; and 0.16±0.01, 0.84±0.01 and 0.19±0.01, respectively). High-fat feeding resulted in significant increases of LFnu and LF/HF ratio (0.28±0.01 and 0.40±0.03, $p<0.05$), accompanied by a significant decrease of HFnu (0.72±0.01, both $p<0.05$). Curcuminoids administration at all doses (30, 60 and 90 mg/kg BW) decreased the high-fat associated changes of power spectral parameters. The LFnu and LF/HF ratio were significantly lower in HD_{30}, HD_{60}, and HD_{90} groups (0.19±0.01 and 0.24±0.01; 0.17±0.01 and 0.20±0.01; and 0.18±0.01 and 0.21±0.01, respectively, all $p<0.05$) than in HDC group. Furthermore, compared with HDC group, the HFnu in HD_{30}, HD_{60}, HD_{90} groups (0.81±0.01, 0.83±0.01 and 0.82±0.01, respectively, all $p<0.05$) were also significant different. By contrast, neither SDNN nor RMSSD was affected by high-fat feeding alone or with curcuminoids administration. SDNN and RMSSD in HDC group were not significantly different (3.91±0.36 and 3.42±0.61, $p>0.05$) from the values presented in NDC group. Compared with HDC group, SDNN and RMSSD in HD_{30}, HD_{60} and HD_{90} groups [(4.23±0.62 and 4.11±0.64), (3.87±0.57 and 3.77±0.82) and (4.11±0.36 and 3.82±0.36), respectively] were also similar.

Table 9 demonstrates the association between HRV and visceral fat, and plasma parameters. The data were collected from both the NDC and HDC groups. Visceral fat mass significantly correlated with LFnu and LF/HF ratio (r = 0.650 and r = 0.616, both $p<0.05$). Furthermore, the plasma free fatty acid levels was also positively related to LFnu and LF/HF ratio (r = 0.589 and r = 0.580, both $p<0.05$). In contrast, a higher level of visceral fat mass was significantly associated with lower HFnu (r = -0.650, $p<0.05$). There was also a significant inverse relationship between plasma free fatty acid levels and HFnu (r = -0.589, $p<0.05$). However, HRV was not

significantly correlated with plasma triglyceride, plasma glucose, and plasma insulin concentrations (r = -0.173 , r = 0.389 and r = -0.442, *p>0.05*).

Table 7 Heart rate variability (HRV) indexes at baseline.

	NDC	**ND_{30}**	**ND_{60}**	**ND_{90}**	**HDC**	**HD_{30}**	**HD_{60}**	**HD_{90}**
SDNN	2.59±0.42	1.22±0.10	2.74±0.89	2.84±0.54	2.77±0.67	2.89±0.65	2.03±0.43	3.77±0.39
RMSSD	1.82±0.27	1.25±0.06	1.77±0.29	2.24±0.34	2.85±0.89	2.95±1.10	2.60±0.46	2.98±0.48
LFnu	0.16±0.00	0.17±0.00	0.17±0.01	0.18±0.01	0.16±0.01	0.18±0.02	0.15±0.01	0.17±0.01
HFnu	0.84±0.00	0.83±0.00	0.83±0.01	0.82±0.01	0.84±0.01	0.82±0.02	0.85±0.01	0.83±0.01
LF/HF ratio	0.19±0.01	0.21±0.00	0.21±0.01	0.22±0.01	0.20±0.01	0.21±0.03	0.17±0.01	0.20±0.01

SDNN; standard deviation of all RR intervals, RMSSD; square root of the mean of the sum of the squares of differences between adjacent RR interval, LFnu; low frequency power in normalized units, HFnu; high frequency power in normalized units. Each value is mean ± SE from 7-12 rats. NDC; normal diet control, ND_{30}; normal diet + curcuminoids at dose of 30 mg/kg BW, ND_{60} ; normal diet + curcuminoids at dose of 60 mg/kg BW, ND_{90} ; normal diet + curcuminoids at dose of 90 mg/kg BW, HDC; high-fat diet control, HD_{30}; high-fat diet + curcuminoids at dose of 30 mg/kg BW, HD_{60} ; high-fat diet + curcuminoids at dose of 60 mg/kg BW, HD_{90} ; high-fat diet + curcuminoids at dose of 90 mg/kg BW.

Table 8 Effect of curcuminoids administration on heart rate variability (HRV) indexes in high-fat diet induced obese rats.

	NDC	**ND_{30}**	**ND_{60}**	**ND_{90}**	**HDC**	**HD_{30}**	**HD_{60}**	**HD_{90}**
SDNN	3.41±0.49	3.54±0.45	2.73±0.31	4.37±0.51 [c]	3.91±0.36	4.23±0.62	3.87±0.57	4.11±0.36
RMSSD	2.42±0.25	2.20±0.22	2.14±0.14	4.23±0.56 [a,b,c]	3.42±0.61	4.11±0.64	3.77±0.82	3.82±0.36
LFnu	0.17±0.01	0.18±0.00	0.18±0.01	0.16±0.01	0.28±0.01 [a]	0.19±0.01 [e]	0.17±0.01 [e,f]	0.18±0.01 [e]
HFnu	0.83±0.01	0.82±0.00	0.82±0.01	0.84±0.01	0.72±0.01 [a]	0.81±0.01 [e]	0.83±0.01 [e,f]	0.82±0.01 [e]
LF/HF ratio	0.20±0.01	0.21±0.01	0.22±0.01	0.19±0.01	0.40±0.03 [a]	0.24±0.01 [e]	0.20±0.01 [e]	0.21±0.01 [e]

SDNN; standard deviation of all RR intervals, RMSSD; square root of the mean of the sum of the squares of differences between adjacent RR interval, LFnu; low frequency power in normalized units, HFnu; high frequency power in normalized units. Each value is mean ± SE from 7-12 rats. NDC; normal diet control, ND_{30}; normal diet + curcuminoids at dose of 30 mg/kg BW, ND_{60} ; normal diet + curcuminoids at dose of 60 mg/kg BW, ND_{90} ; normal diet + curcuminoids at dose of 90 mg/kg BW, HDC; high-fat diet control, HD_{30}; high-fat diet + curcuminoids at dose of 30 mg/kg BW, HD_{60} ; high-fat diet + curcuminoids at dose of 60 mg/kg BW, HD_{90} ; high-fat diet + curcuminoids at dose of 90 mg/kg BW. [a] ; significantly different from NDC group, [e] ; significantly different from HDC group, [f] ; significantly different from HD_{30} group, $p<0.05$.

Table 9 Correlation between heart rate variability (HRV) and visceral fat and plasma parameters in rats consumed normal diets or high-fat diets for 12 weeks.

	Heart Rate variability		
	LFnu	**HFnu**	**LF/HF ratio**
Visceral Fat, g	0.650 **	-0.650 **	0.616 *
Plasma free fatty acid, mmol/l	0.589 *	-0.589 *	0.580 *
Plasma triglyceride, mg/dl	-0.168	0.168	-0.173
Plasma glucose, mg/dl	0.423	-0.423	0.389
Plasma insulin, ng/ml	-0.423	0.423	-0.442

LFnu; Low frequency power in normalized units, HFnu; High frequency power in normalized units. * $p<0.05$, ** $p<0.01$.