CHAPTER IV

RESULTS

Part I: The effects of high fat and high sucrose diet.

Effect of high fat and high sucrose diet on food intake

Through 20 weeks of induction by high fat and high sucrose diet, the food intake in all HFHS groups was statistically significant less than that of the C group ($P < 0.001$). In the C group, the average value of food intake for each animal was as follows: weeks 4 (3.06 ± 0.03 g), weeks 8 (3.12 ± 0.03 g), weeks 12 (3.07 ± 0.05 g), weeks 16 (3.19 ± 0.04 g) and weeks 20 (3.21 ± 0.03 g). Food intake for the HFHS-C group was as follows: weeks 4 (2.25 ± 0.09 g), weeks 8 (2.26 ± 0.03 g), weeks 12 (2.25 ± 0.04 g), weeks 16 (2.44 ± 0.04 g) and weeks 20 (2.73 ± 0.04 g). Additionally, there was no statistically significant difference of food intake in the HFHS+KPE10 and HFHS+KPE100 groups when compared to the HFHS-C group (Table 2).

Effect of high fat and high sucrose diet on body weight

The body weight at the beginning of the experiment was not significantly different in the HFHS-C (19.16 ± 0.61 g), the HFHS+KPE10 (19.13 ± 0.28 g) and the HFHS+KPE100 (19.11 ± 0.21 g) groups when compared to the C group (18.89 ± 0.37 g). In the first 12 weeks of induction with high fat and high sucrose diet, the body weight in all HFHS groups was statistically significant less than that of the C group as shown in table 3 ($P < 0.05$). Nevertheless, the body weight in all HFHS groups was statistically significant higher than the C group in the twentieth week (33.03 ± 0.90, 32.61 ± 0.69, and 32.71 ± 0.50 g in the HFHS-C, the HFHS+KPE10 and the HFHS+KPE100, respectively *vs* 30.97 ± 0.50; $P < 0.05$)(Figure 10).

Table 2 The average of the food intake of mice (g/day/mouse) fed with high fat and high sucrose diet during 20 weeks

Group	Normal diet			High fat and high sucrose diet		
	C	C+KPE10	C+KPE100	HFHS-C	HFHS+KPE10	HFHS+KPE100
	(n = 10)	(n = 10)	(n = 10)	(n = 7)	(n = 10)	(n = 13)
Week 4	3.06 ± 0.03	3.04 ± 0.07	3.06 ± 0.05	2.25 ± 0.09$^{\#\#}$	2.21 ± 0.07$^{\#\#}$	2.28 ± 0.03$^{\#\#}$
Week 8	3.12 ± 0.03	3.16 ± 0.03	3.17 ± 0.02	2.26 ± 0.03$^{\#\#}$	2.24 ± 0.01$^{\#\#}$	2.25 ± 0.08$^{\#\#}$
Week 12	3.07 ± 0.05	3.11 ± 0.03	3.12 ± 0.03	2.25 ± 0.04$^{\#\#}$	2.25 ± 0.05$^{\#\#}$	2.26 ± 0.04$^{\#\#}$
Week 16	3.19 ± 0.04	3.21 ± 0.03	3.21 ± 0.02	2.44 ± 0.04$^{\#\#}$	2.43 ± 0.05$^{\#\#}$	2.38 ± 0.04$^{\#\#}$
Week 20	3.21 ± 0.03	3.22 ± 0.03	3.28 ± 0.03	2.73 ± 0.04$^{\#\#}$	2.69 ± 0.04$^{\#\#}$	2.75 ± 0.04$^{\#\#}$

Note: The data represents the mean ± SEM $^{\#\#}P < 0.01$ compared to the C (one-way ANOVA with post-hoc LSD test).

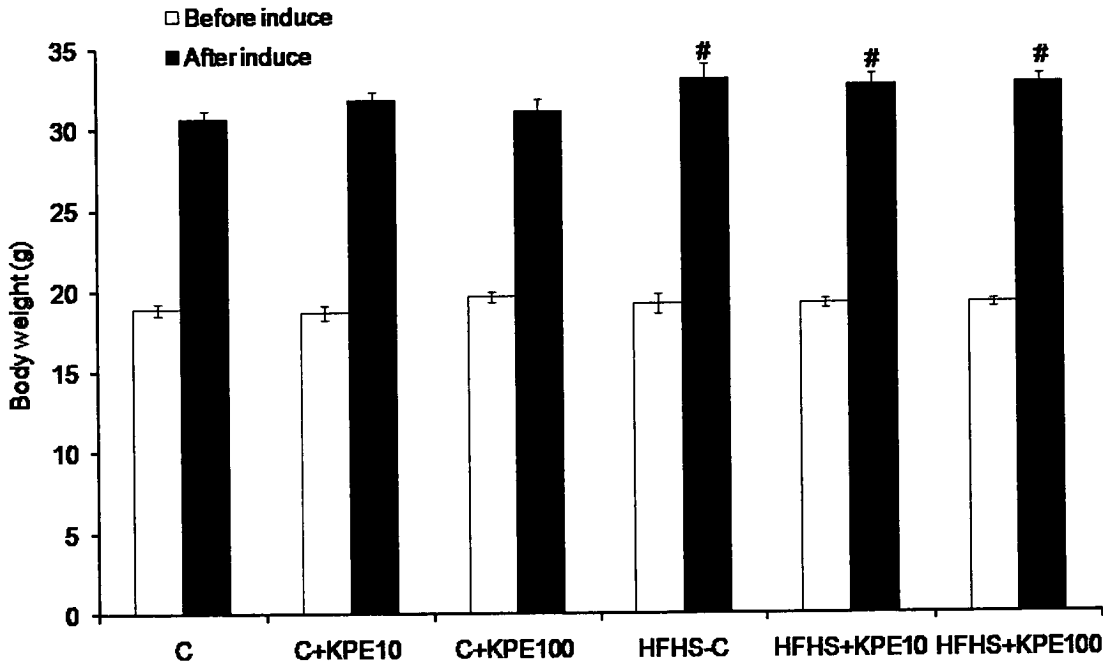

Figure 10 The body weight of mice fed with high fat and high sucrose diet for 20 weeks. Each bar represents the mean ± SEM (n = 10 except HFHS-C; n = 7 and HFHS+KPE100; n=13). $^{\#}P < 0.05$ compared to the C (one-way ANOVA with post-hoc LSD test).

Table 3 The body weight (g) of mice fed with high fat and high sucrose diet for 20 weeks

Group	Normal diet			High fat and high sucrose diet		
	C	C+KPE10	C+KPE100	HFHS-C	HFHS+KPE10	HFHS+KPE100
	(n = 10)	(n = 10)	(n = 10)	(n = 7)	(n = 10)	(n = 13)
Week 0	18.89 ± 0.37	18.65 ± 0.44	19.61 ± 0.34	19.16 ± 0.61	19.13 ± 0.28	19.11 ± 0.21
Week 4	25.52 ± 0.37	25.59 ± 0.47	25.32 ± 0.50	23.30 ± 0.53$^{\#}$	23.48 ± 0.41$^{\#}$	23.15 ± 0.31$^{\#}$
Week 8	28.39 ± 0.33	28.72 ± 0.45	27.90 ± 0.60	26.28 ± 0.72$^{\#}$	25.80 ± 0.44$^{\#}$	26.67 ± 0.35$^{\#}$
Week 12	29.40 ± 0.43	29.87 ± 0.48	29.53 ± 0.64	27.69 ± 0.84$^{\#}$	27.57 ± 0.49$^{\#}$	27.59 ± 0.52$^{\#}$
Week 16	30.63 ± 0.49	30.86 ± 0.44	30.22 ± 0.71	30.34 ± 0.85	29.92 ± 0.59	30.99 ± 0.59
Week 20	30.97 ± 0.50	31.52 ± 0.52	31.01 ± 0.81	33.03 ± 0.90$^{\#}$	32.61 ± 0.69$^{\#}$	32.71 ± 0.50$^{\#}$

Note: The data represents the mean ± SEM $^{\#}P < 0.05$ compared to the C (one-way ANOVA with post-hoc LSD test).

Effect of high fat and high sucrose diet on fasting blood glucose concentration

At the beginning of the experiment, the blood glucose concentration was not significantly different in the HFHS-C (83.86 ± 2.25 mg/dl), the HFHS+KPE10 (83.90 ± 1.62 mg/dl) and the HFHS+KPE100 (83.38 ± 2.44 mg/dl) groups when compared to the C group (84.70 ± 1.89 mg/dl). The blood glucose concentration after 20 weeks of induction with high fat and high sucrose diet was statistically significant higher in all HFHS groups (168.43 ± 9.26 mg/dl, 158.60 ± 9.42 mg/dl and 165.38 ± 11.39 mg/dl in the HFHS-C, the HFHS+KPE10 and the HFHS+KPE100, respectively) than the C group (117.20 ± 6.70 mg/dl; $P < 0.001$) (Figure 11).

Effect of high fat and high sucrose diet on systolic blood pressure

In this study, the systolic blood pressure was not examined at the beginning of the experiment because it was unable to detect in the 3-weeks old mice. At the end of the 20th week of the high fat and high sucrose diet induction, the statistical analysis of the systolic blood pressure demonstrated that there was no significant difference between the experimental groups (111.24 ± 3.12 *vs* 113.00 ± 2.47 mmHg in HFHS-C and C groups, respectively; $P = 0.658$) (Figure 12).

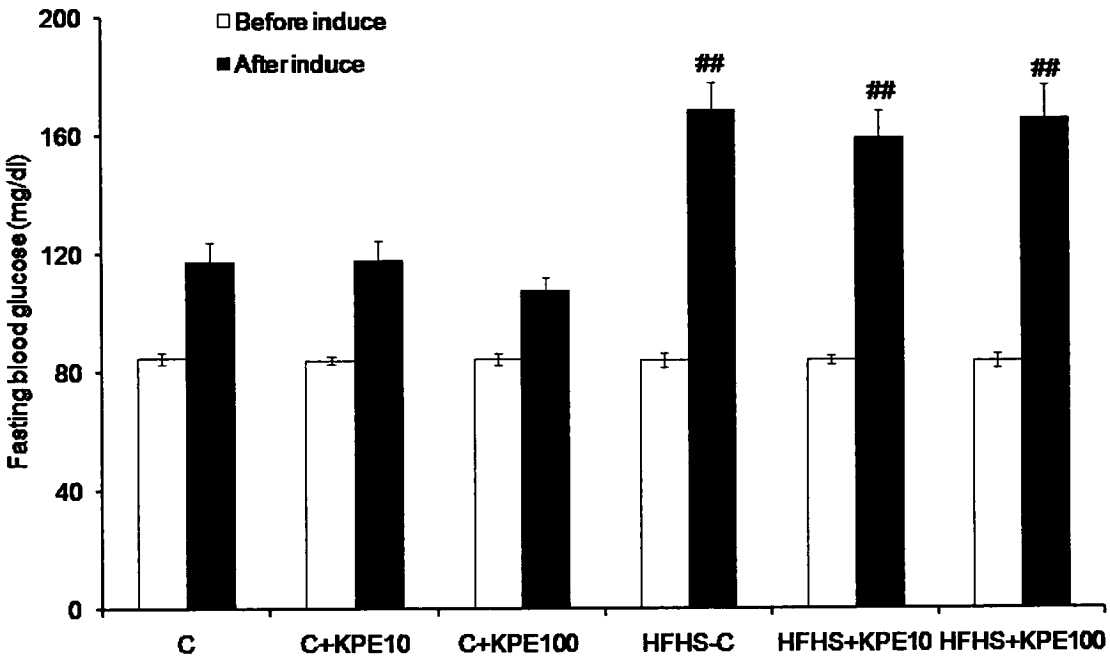

Figure 11 The fasting blood glucose concentration of mice fed with high fat and high sucrose diet for 20 weeks. The data are expressed as the blood glucose concentration before and after induction. Each bar represents the mean ± SEM (n = 10 except HFHS-C; n = 7 and HFHS+KPE100; n=13). $^{\#\#}P < 0.01$ compared to the C (one-way ANOVA with post-hoc LSD test).

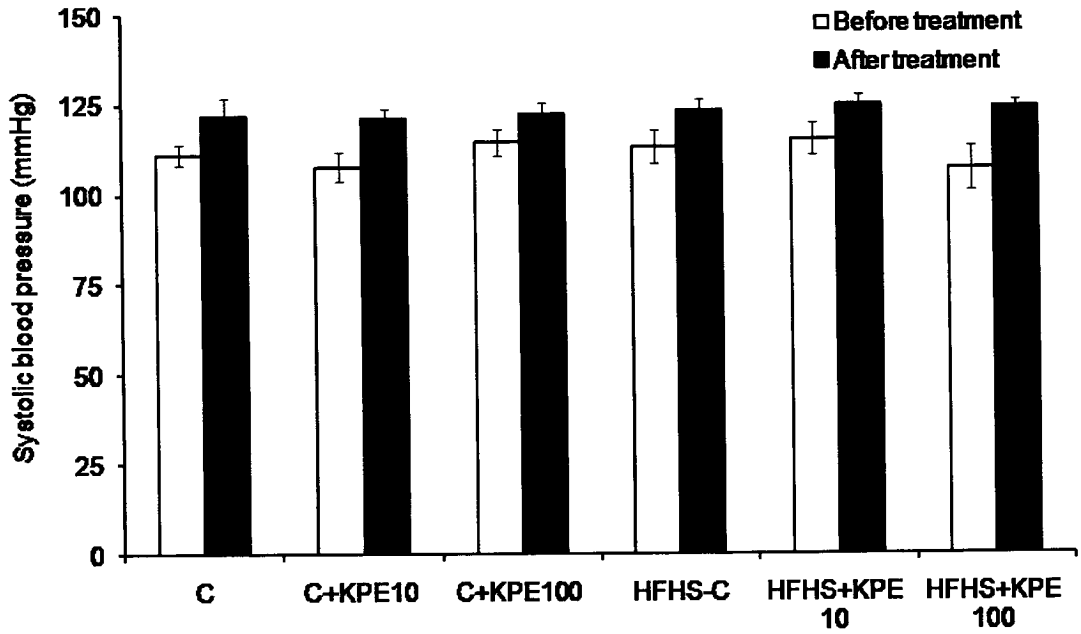

Figure 12 The systolic blood pressure of mice administration with *K. parviflora* extract for 8 weeks. The data are expressed as the systolic blood pressure before and after treatment. Each bar represents the mean ± SEM (n = 10 except HFHS-C; n = 7 and HFHS+KPE100; n=13) (one-way ANOVA with post-hoc LSD test).

Part II: The effects of *K. parviflora* extract on mice fed with high fat and high sucrose diet.

Effect of *K. parviflora* extract on systolic blood pressure

The systolic blood pressure was evaluated after 8 weeks of *K. parviflora* extract administration by tail cuff method. The systolic blood pressure no significant differences were found among groups (122.00 ± 4.84, 121.17 ± 2.74 and 122.33 ± 2.93 mmHg in the C, C+KPE10 and C+KPE100 groups; 123.14 ± 2.96, 124.67 ± 2.56 and 123.70 ± 2.15 mmHg in the HFHS-C, HFHS+KPE10 and HFHS+KPE100 groups, respectively) (Figure 12).

Effect of *K. parviflora* extract on food intake

Throughout 8 weeks of *K. parviflora* treatment, the food intakes in all HFHS groups were statistically significant less than the C groups. For the first 4 weeks the average value of the food intake was as follows 2.58 ± 0.04 g (HFHS-C), 2.61 ± 0.05 g (HFHS+KPE10), 2.70 ± 0.06 g (HFHS+KPE100), 3.00 ± 0.05 g (C), 3.02 ± 0.04 g (C+KPE10) and 3.07 ± 0.07 g (C+KPE100). For the 4 weeks later, the results were as same as the first 4 weeks which were 2.60 ± 0.04 g (HFHS-C), 2.58 ± 0.04 g (HFHS+KPE10), 2.64 ± 0.04 g (HFHS+KPE100), 3.01 ± 0.04 g (C), 2.98 ± 0.03 g (C+KPE10) and 3.00 ± 0.05 g (C+KPE100) (Figure 13). In addition, the food intakes of mice in *K. parviflora* treated groups were not statistically significant difference when compared with its own control groups. The study revealed that food intake patterns of mice before and after treatment with *K. parviflora* were the same.

Effect of *K. parviflora* extract on body weight

There was no statistically significant difference in body weight of all *K. parviflora* treated groups when compared to its own control groups as shown in figure 14: 33.44 ± 0.55 g (HFHS+KPE10), 34.37 ± 0.72 g (HFHS+KPE100), 33.81 ± 0.89 g (HFHS-C), 31.96 ± 0.51 g (C+KPE10), 31.74 ± 0.77g (C+KPE100) and 31.51 ± 0.44 g (C). The finding indicated that *K. parviflora* extract did not affect on body weight change in both normal as well as high fat and high sucrose diet mice.

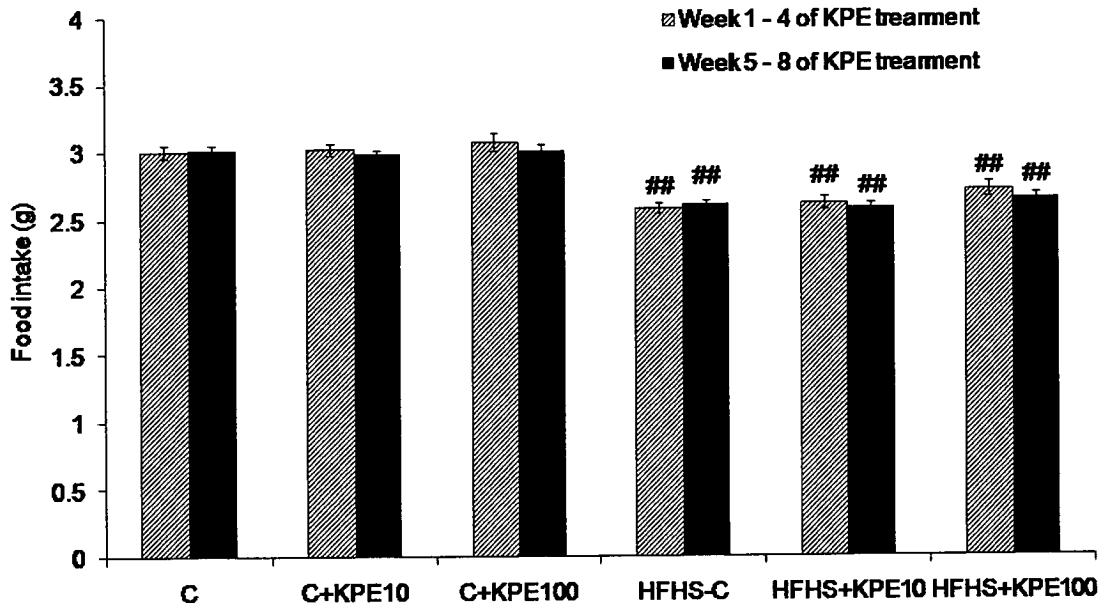

Figure 13 The food intake of mice during administration with *K. parviflora* extract for 8 weeks. The data are expressed as the average value of food intake at week 1-4 and week 5-8 of treatment. Each bar represents the mean ± SEM (n = 10 except HFHS-C; n = 7 and HFHS+KPE100; n=13). $^{\#\#}P < 0.01$ compared to the C (one-way ANOVA with post-hoc LSD test).

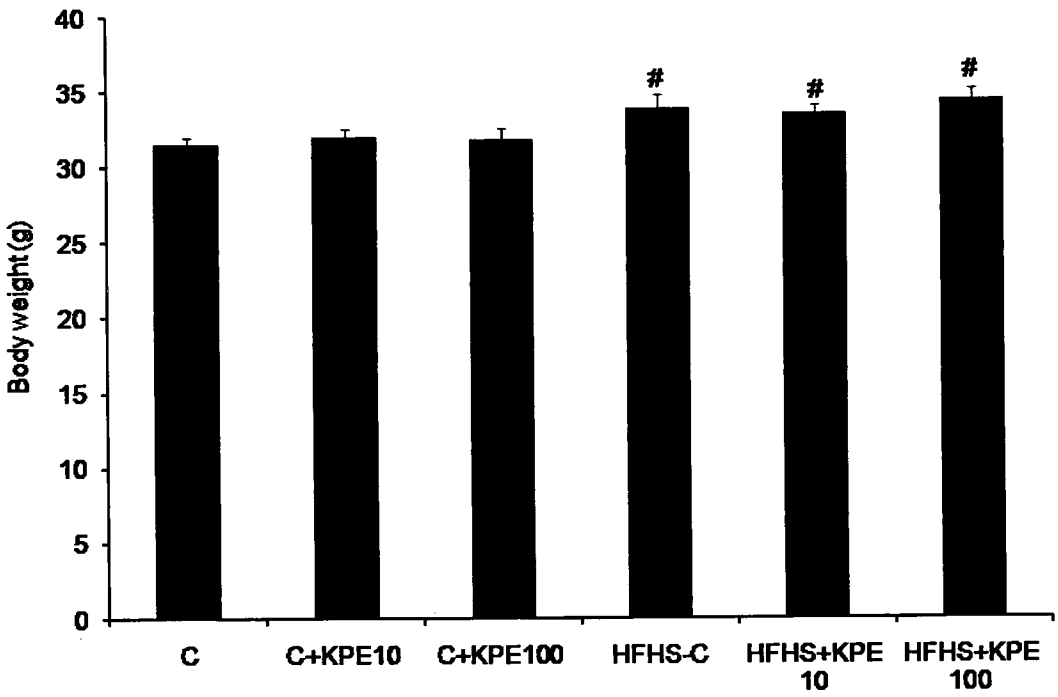

Figure 14 The body weight of mice during administration with *K. parviflora* extract for 8 weeks. Each bar represents the mean ± SEM (n = 10 except HFHS-C; n = 7 and HFHS+KPE100; n=13). $^{\#}P < 0.05$ compared to the C (one-way ANOVA with post-hoc LSD test).

Effect of *K. parviflora* extract on fasting blood glucose

The fasting blood glucose was determined before and after 8 weeks of *K. parviflora* administration by glucose oxidase method.

Before administration, the results indicated that fasting blood glucose level in the HFHS-C group was significant higher than the C group (168.43 ± 9.25 mg/dl *vs* 117.20 ± 6.70 mg/dl; $P < 0.001$). There was no statistically significant difference in blood glucose concentration in both HFHS treated groups when compared to the HFHS-C group (158.60 ± 9.42 and 165.38 ± 12.39 mg/dl *vs* 168.43 ± 9.25 mg/dl in HFHS+KPE10, HFHS+KPE100 and HFHS-C group, respectively).

At the end of the *K. parviflora* treatment, the fasting blood glucose level were statistically significant decreased in the HFHS treated groups (116.30 ± 7.66 mg/dl in the HFHS+KPE10 and 118.54 ± 2.74 mg/dl in the HFHS+KPE100; $P < 0.001$) when compared to the HFHS-C group (172.43 ± 9.07 mg/dl). On the contrary, there was no significant difference between the C treated group and the C group (101.10 ± 4.50 and 103.70 ± 3.62 mg/dl *vs* 112.30 ± 4.71 mg/dl in C+KPE10, C+KPE100 and C, respectively) (Figure 15).

Effect of acute *K. parviflora* extract on oral glucose tolerance test

The oral glucose tolerance test was evaluated at first day of *K. parviflora* administration by glucose oxidase method. The result revealed that blood glucose level in the HFHS-C group was significant higher than the C group in all time points ($P < 0.05$). Blood glucose level significantly decreased in the HFHS+KPE10 group at 120 min only when compared to the HFHS-C group ($P < 0.05$) (Figure 16, 17 and Table 4). In contrast, blood glucose level in the control and control treated groups were not different in all time points.

Effect of subchronic *K. parviflora* extract on oral glucose tolerance test

The oral glucose tolerance test was assessed at the end of *K. parviflora* administration again. The result indicated that there was significant increase of blood glucose level in the HFHS-C group when compared to the C group ($P < 0.001$) at all time points. In contrast, which mean that *K. parviflora* extract had no significant effect on blood glucose in both unhealthy and normal mice (Figure 18, 19 and Table 5).

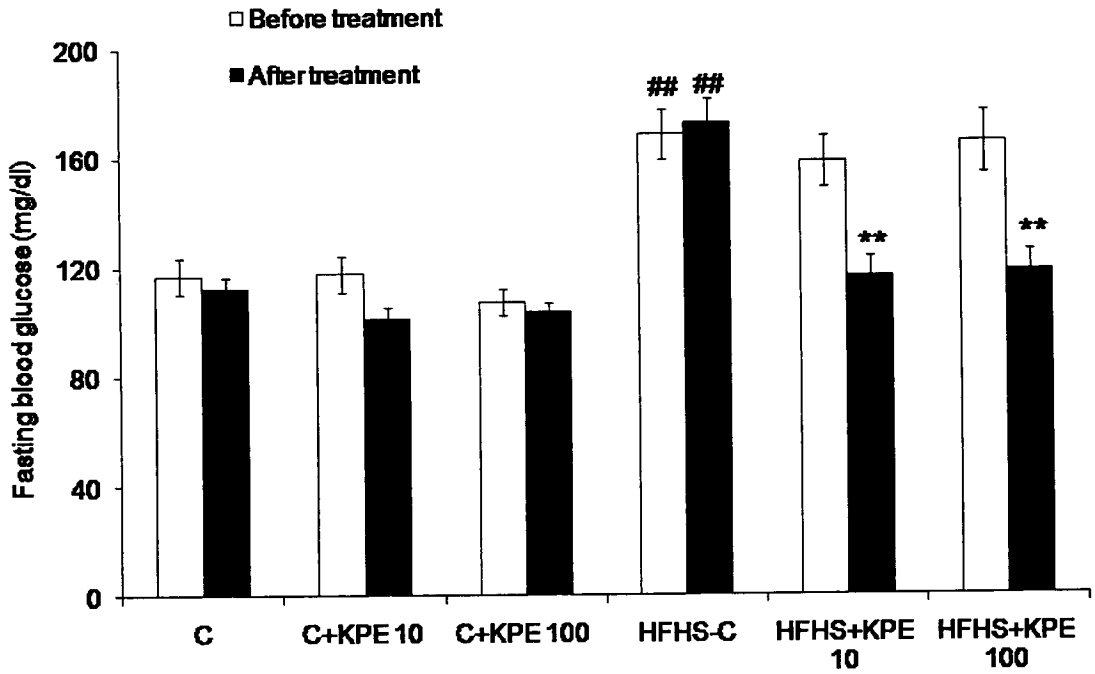

Figure 15 The fasting blood glucose concentration of mice before and after treatment with *K. parviflora* extract for 8 weeks. Each bar represents the mean ± SEM (n = 10 except HFHS-C; n = 7 and HFHS+KPE100; n=13). $^{\#\#}P < 0.01$ compared to the C, $^{**}P < 0.01$ compared to HFHS-C (one-way ANOVA with post-hoc LSD test).

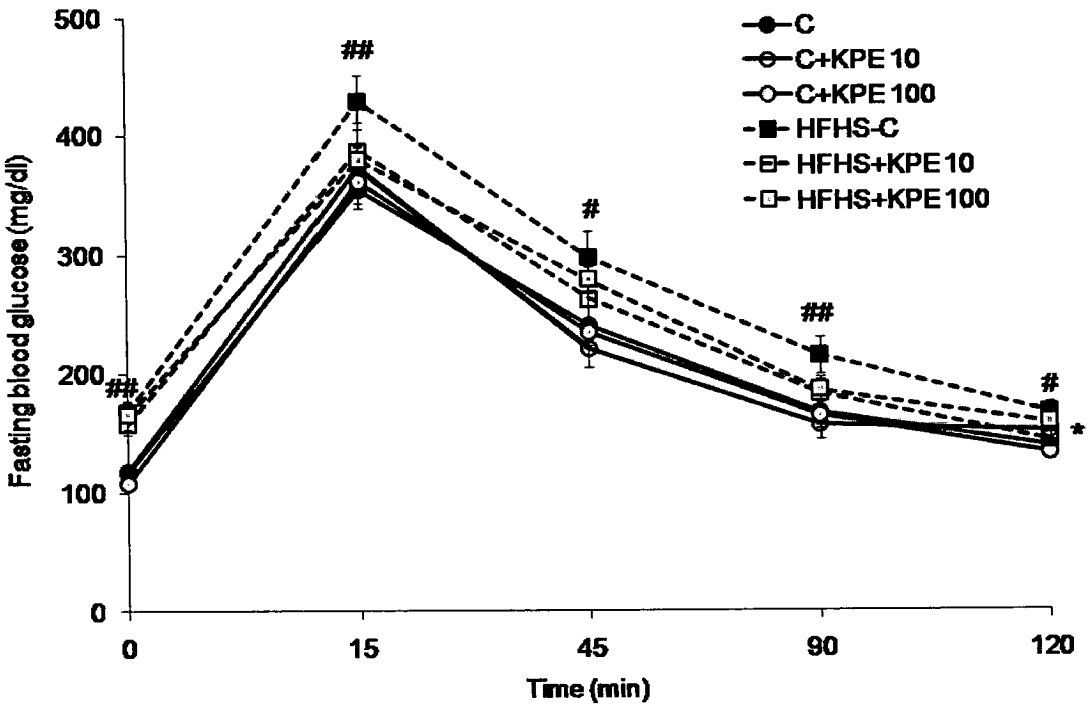

Figure 16 The oral glucose tolerance test of mice at the first day of *K. parviflora* administration (acute administration). Each bar represents the mean ± SEM (n = 10 except HFHS-C; n = 7 and HFHS+KPE100; n=13). $^{\#}P < 0.05$ or $^{\#\#}P < 0.01$ compared to the C, $^{*}P < 0.05$ compared to HFHS-C (one-way ANOVA with post-hoc LSD test).

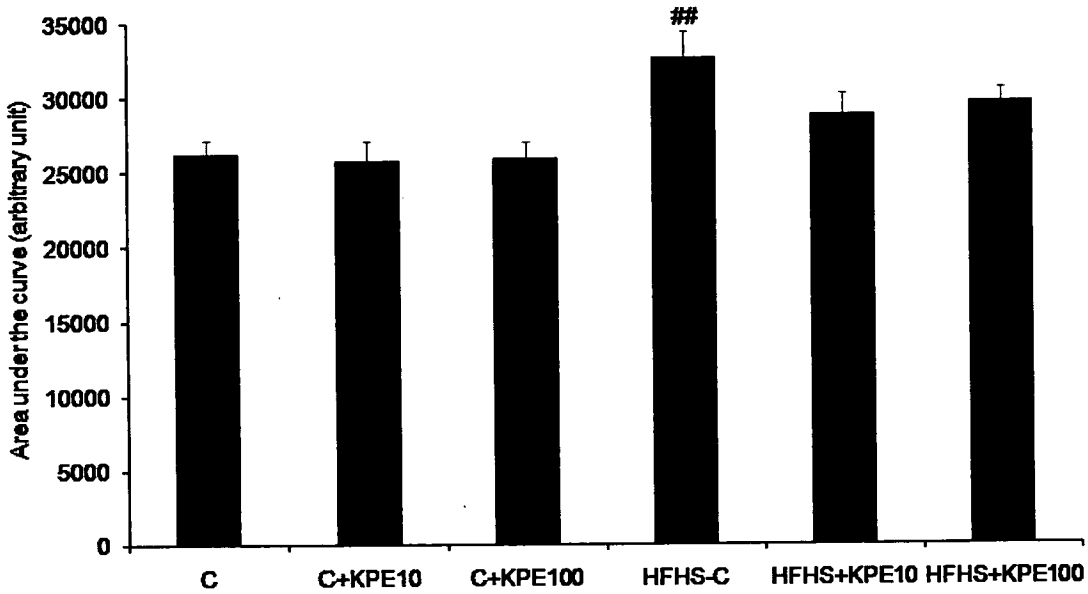

Figure 17 The areas under the curve of mice at the first day of *K. parviflora* administration (acute administration). Each bar represents the mean ± SEM (n = 10 except HFHS-C; n = 7 and HFHS+KPE100; n=13). $^{\#\#}P < 0.01$ compared to the C, (one-way ANOVA with post-hoc LSD test).

Table 4 The fasting blood glucose (mg/dl) in the oral glucose tolerance test of mice at the first day of *K. parviflora* administration (acute administration)

Group	Normal diet			High fat and high sucrose diet		
	C	C+KPE10	C+KPE100	HFHS-C	HFHS+KPE10	HFHS+KPE100
	(n = 10)	(n = 10)	(n = 10)	(n = 7)	(n = 10)	(n = 13)
0 min	117.20 ± 6.70	117.80 ± 6.74	107.20 ± 4.79	168.43 ± 9.26$^{\#\#}$	158.60 ± 9.42	165.38 ± 11.39
15 min	354.20 ± 15.13	373.80 ± 18.00	361.50 ± 18.08	428.43 ± 22.66$^{\#\#}$	387.20 ± 23.59	379.70 ± 10.93
45 min	240.10 ± 16.39	221.00 ± 15.63	234.50 ± 8.79	297.14 ± 22.49$^{\#}$	262.70 ± 16.52	278.69 ± 11.09
90 min	167.40 ± 4.92	156.60 ± 11.87	164.80 ± 10.61	214.86 ± 15.49$^{\#\#}$	183.00 ± 13.87	186.00 ± 8.58
120 min	138.50 ± 4.27	150.90 ± 9.26	132.90 ± 4.75	167.00 ± 8.38$^{\#\#}$	143.50 ± 8.36*	158.54 ± 6.67

Note: The data represents the mean ± SEM $^{\#}P < 0.05$ or $^{\#\#}P < 0.01$ compared to the C, $^{*}P < 0.05$ compared to HFHS-C (one-way ANOVA with post-hoc LSD test).

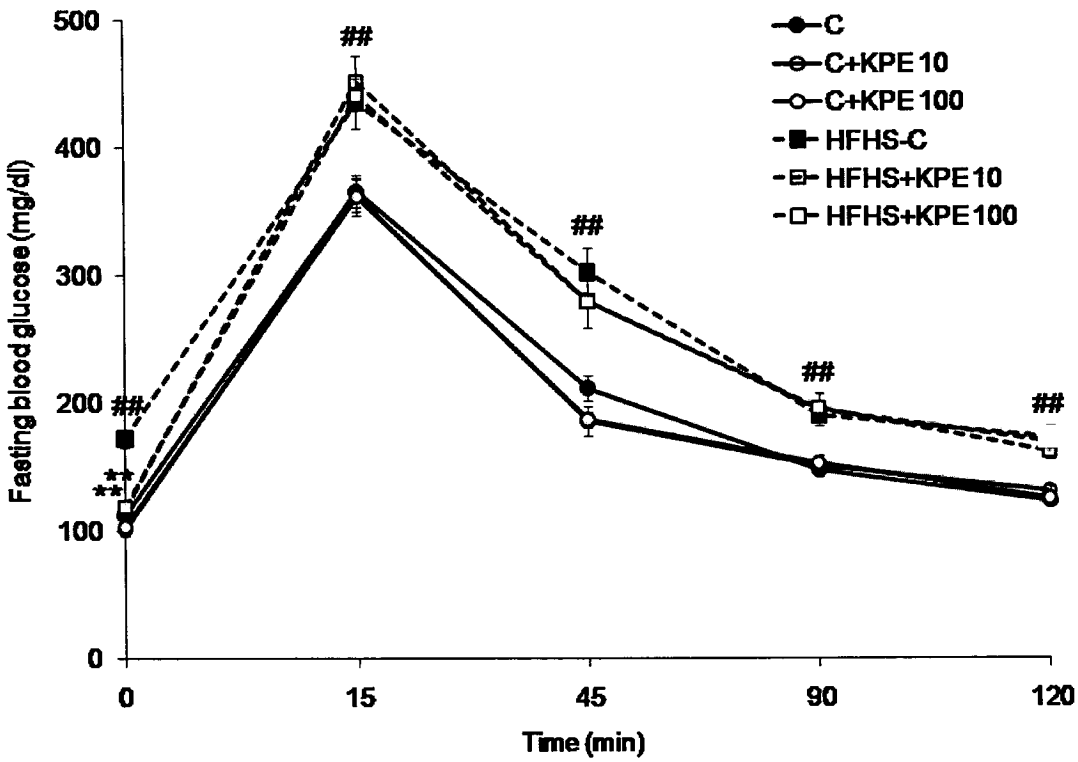

Figure 18 The oral glucose tolerance test of mice after administration with *K. parviflora* extract for 8 weeks. Each bar represents the mean ± SEM (n = 10 except HFHS-C; n = 7 and HFHS+KPE100; n=13). $^{\#\#}P < 0.01$ compared to the C, $^{**}P < 0.01$ compared to HFHS-C (one- way ANOVA with post-hoc LSD test).

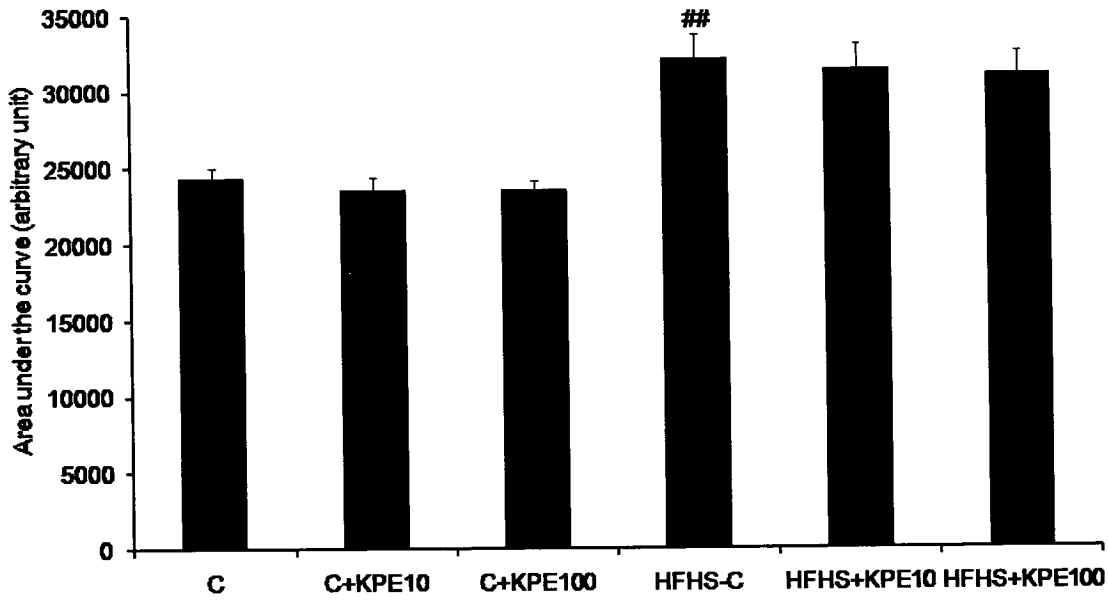

Figure 19 The areas under the curve of mice after administration with *K. parviflora* extract for 8 weeks. Each bar represents the mean ± SEM (n = 10 except HFHS-C; n = 7 and HFHS+KPE100; n=13). $^{\#\#}P < 0.01$ compared to the C, (one-way ANOVA with post-hoc LSD test).

Table 5 The fasting blood glucose (mg/dl) in the oral glucose tolerance test of mice after administration with *K. parviflora* extract for 8 weeks

Group	Normal diet			High fat and high sucrose diet		
	C	C+KPE10	C+KPE100	HFHS-C	HFHS+KPE10	HFHS+KPE100
	(n = 10)	(n = 10)	(n = 10)	(n = 7)	(n = 10)	(n = 13)
0 min	112.30 ± 4.71	101.10 ± 4.50	103.70 ± 3.62	172.43 ± 9.08$^{\#\#}$	116.30 ± 7.66**	118.54 ± 7.27**
15 min	365.80 ± 12.17	362.90 ± 13.12	360.80 ± 13.87	434.57 ± 23.71$^{\#\#}$	451.50 ± 20.50	439.85 ± 13.73
45 min	211.10 ± 9.58	185.50 ± 11.49	187.00 ± 5.77	302.00 ± 23.57$^{\#\#}$	279.50 ± 20.40	278.69 ± 16.82
90 min	147.00 ± 5.08	150.40 ± 5.60	152.70 ± 6.43	190.00 ± 6.37$^{\#\#}$	194.60 ± 12.66	195.38 ± 14.62
120 min	122.80 ± 3.37	130.40 ± 4.63	125.60 ± 3.81	172.57 ± 5.88$^{\#\#}$	168.80 ± 13.46	161.38 ± 9.93

Note: The data represents the mean ± SEM $^{\#\#}P < 0.01$ compared to the C, $^{**}P < 0.01$ compared to HFHS-C (one-way ANOVA with post hoc LSD test).

Effect of *K. parviflora* extract on serum cholesterol, triglycerides and high-density lipoprotein cholesterol

The serum cholesterol, triglycerides and high-density lipoprotein cholesterol concentration were evaluated at the end of *K. parviflora* treatment.

The result revealed that the serum cholesterol concentration in the HFHS-C group was significant higher than the C group (222.43 ± 2.15 *vs* 93.90 ± 2.96 mg/dl; $P < 0.001$). The serum high-density lipoprotein cholesterol concentration in the HFHS-C group was significant lower than the C group (35.86 ± 3.36 *vs* 79.00 ± 4.26 mg/dl; $P < 0.001$). Whereas, there was no statistically significant difference of triglycerides concentration in the HFHS-C group when compared to the C group (87.71 ± 3.33 *vs* 94.20 ± 2.69 mg/dl; $P = 0.412$).

After *K. parviflora* treatment, the serum cholesterol concentration statistically significant decreased in both HFHS treated groups when compared to the HFHS-C group (142.80 ± 6.78, 123.92 ± 3.43 and 222.43 ± 2.15 mg/dl in the HFHS+KPE10, HFHS+KPE100 and HFHS-C groups, respectively; $P < 0.001$). Serum triglycerides concentration significant decreased in the HFHS+KPE10 group only when compared to the HFHS-C group (70.30 ± 5.68 *vs* 87.71 ± 3.33 mg/dl; $P < 0.05$). The high-density lipoprotein cholesterol concentrations were significantly increased in both HFHS treated groups when compared to the HFHS-C group (83.30 ± 5.03 mg/dl in the HFHS+KPE10, 70.92 ± 3.20 mg/dl in the HFHS+KPE100 *vs* 35.86 ± 3.36 mg/dl in the HFHS-C; $P < 0.001$).

It was note that, *K. parviflora* extract did not alter the serum cholesterol, triglycerides and high-density lipoprotein cholesterol concentration in mice fed with normal diet (Table 6).

Table 6 The serum cholesterol, triglycerides and high-density lipoprotein cholesterol concentration (mg/dl) of mice after administration with *K. parviflora* extract for 8 weeks

Group	Normal diet			High fat and high sucrose diet		
	C	C+KPE10	C+KPE100	HFHS-C	HFHS+KPE10	HFHS+KPE100
	(n = 10)	(n = 10)	(n = 10)	(n = 7)	(n = 10)	(n = 13)
Cholesterol	93.90 ± 2.96	100.40 ± 5.38	91.90 ± 3.70	222.43 ± 2.15$^{\#\#}$	142.80 ± 6.78**	123.92 ± 3.43**
Triglycerides	94.20 ± 2.69	88.60 ± 5.00	85.80 ± 6.70	87.71 ± 3.33	70.30 ± 5.68*	77.85 ± 4.66
HDL-C	79.00 ± 4.20	68.30 ± 2.61	73.10 ± 4.39	35.86 ± 3.36$^{\#\#}$	83.30 ± 5.03**	70.92 ± 3.20**

Note: The data represents the mean ± SEM $^{\#\#}P < 0.01$ compared to the C, $^{*}P < 0.05$ or $^{**}P < 0.01$ compared to HFHS-C (one-way ANOVA with post-hoc LSD test)

Effect of *K. parviflora* extract on visceral fat accumulation and adipocyte morphology

The results of this study indicated that the visceral fat mass increased significantly in the HFHS-C group when compared to the C group (0.050 ± 0.006 *vs* 0.013 ± 0.001 g; $P < 0.001$) (Figure 20). Moreover, the morphology of adipocytes, diameter of fat cells in the HFHS group was larger than that of the C group (121.44 ± 2.43 *vs* 87.98 ± 2.31 μm; $P < 0.001$). The result showed that there was hypertrophy of adipocytes in the HFHS-C group (Figure 21 A, D).

After *K. parviflora* treatment for 8 weeks, the visceral fat mass decreased significantly in the HFHS+KPE10 group only (0.032 ± 0.005 g; $P < 0.01$) when compared to the HFHS-C group (0.050 ± 0.006 g) (Figure 20). Nevertheless, the visceral fat mass in the HFHS+KPE100 group slightly decreased but not significant difference when compared with the HFHS-C group (0.044 ± 0.006 *vs* 0.050 ± 0.006 g; $P = 0.36$). Additionally, in both HFHS treated groups, the adipocytes diameter was less than that of the HFHS-C group (82.26 ± 1.48 μm; $P < 0.001$ in the HFHS+KPE10 and 115.29 ± 2.64 μm; $P < 0.05$ in the HFHS+KPE100, respectively) (Figure 21 B-F).

It was noted that, *K. parviflora* extract did not change the visceral fat accumulation and the size of fat cells in mice fed with normal diet.

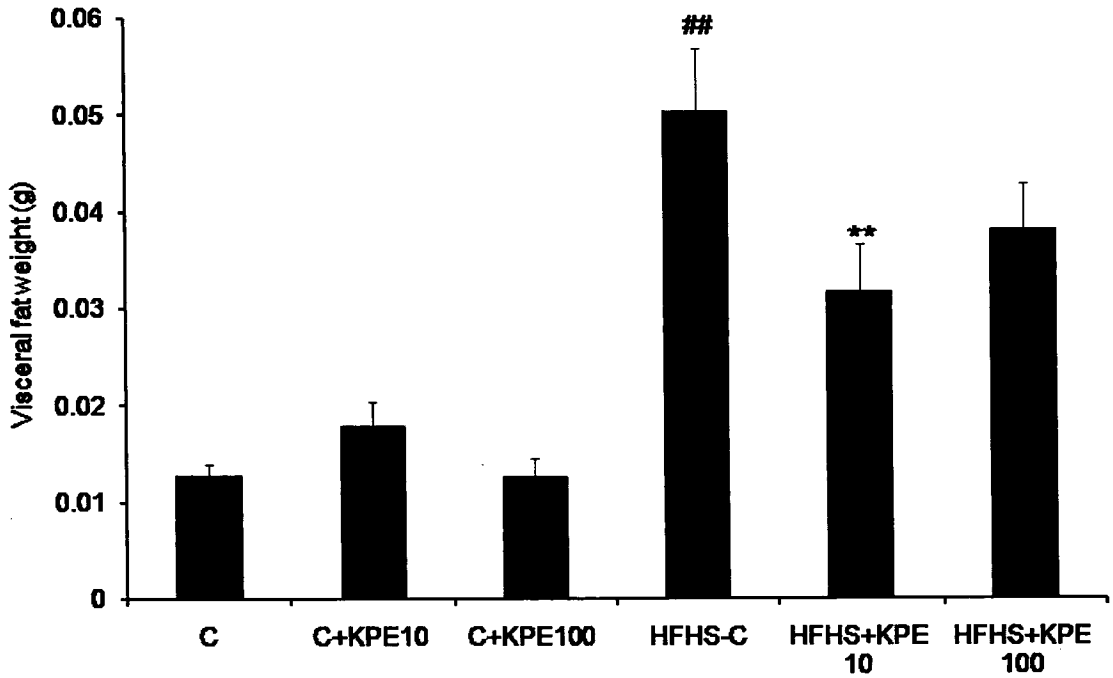

Figure 20 The visceral fat accumulation in mice that administered *K. parviflora* extract for 8 weeks. Each bar represents the mean ± SEM (n = 10 except HFHS-C; n = 7 and HFHS+KPE100; n=13). $^{\#\#}P < 0.01$ compared to the C, $^{**}P < 0.01$ compared to HFHS-C (one-way ANOVA with post-hoc LSD test).

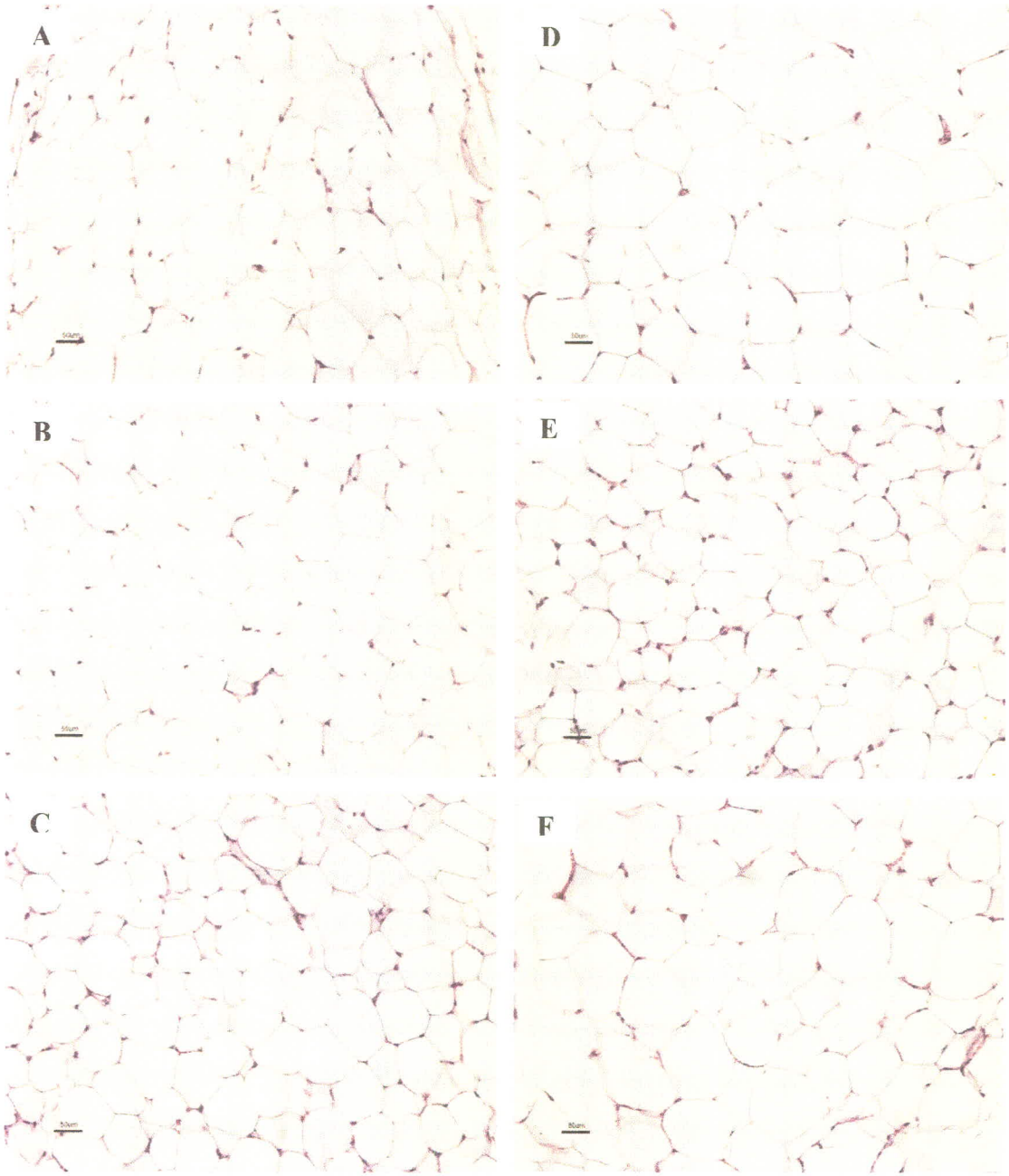

Figure 21 Microscopic photographs of adipocytes from the visceral fat (40X) after 8 weeks of *K. parviflora* treatment.

Note: Scale bar = 25 µm, A = C group, B = C+KPE10 group, C = C+KPE100 group, D = HFHS-C group, E = HFHS+KPE10 group, F = HFHS+KPE100 group.