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Effects of the Red Garlic Extract for Anti-Obesity and Hypolipidemic in Obese Rats Induced High Fat Diet

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This study tested the anti-obesity and hypolipidemic effects of red garlic extract in obese rats induced by a high fat diet over a period of 4 weeks. Red garlic extract of 15 brix was added in 1, 3, 5 and 7% ratios in diets. The obesity index and body fat content significantly decreased in rats fed a diet with over 3% red garlic extract compared to the control group. There was no significant difference in weight of visceral and epididymal fat in rats fed red garlic extract. Total lipid and triglyceride levels in serum were significantly decreased in a dose-dependent manner, and AI and CRF also fell. ALT and AST activities in groups fed red garlic extract were decreased compared to the control group. Total lipid level in liver tissue of the groups fed 5-7% red garlic extract exhibited a significant decrease compared to the control group. Total cholesterol and triglyceride levels in feces were significantly increased in rats fed a diet with over 5% red garlic extract. Lipid peroxide levels were significantly decreased in the groups fed diets with 5-7% red garlic and antioxidant activity in serum was significantly increased in the group fed a diet with 7% red garlic extract compared to the control group. Our results suggest that red garlic extract could have anti-obesity and hypolipidemic effects for suppressing obesity index and decreasing lipid profiles.

Key words : Anti-obesity, hypolipidemic, red garlic extract

Introduction

As the choice for a high-calorie and high-fat diet increases due to the economic stability and affluence, so does the occurrence of metabolic disease caused by over-weight [27]. Obesity is the condition under which surplus energy is accumulated as lipids, which is attributable to the increase of energy intake and the decrease of consumption. From the balance is lost, obesity or hyperlipidemia develops, leading to a serious disease. In Korea, the social and economic cost incurred by the increase of obese population rises annually. Given that 40% of people who die by circulating system disease including atherosclerosis, cerebro-, cardio-vascular complications, hypertention, diabetes and functional depression of certain organs are obese, it is necessary to take a fundamental measure to improve the situation [47].

Although the drug therapy proved to be effective as a behavior correction therapy used for the control of lipid metabolism in obese, it accompanies side-effects such as headaches, insomnia, and vomiting [29]. Thus, there is a growing attention on the way to improve obesity using phyto-nutrient that does not cause side-effects, among which garlic is known to effective for the control of lipid metabolism and obesity [1,16,24,31,51]. A major bioactive substances of garlic (*Allium sativum* L.) are an organic sulfur compounds containing allicin [11,18,22], which has the cardioprotective function among others [25]. Reportedly in Korea, the bioactive effect is elevated through the heat-treatment which turns fresh garlic into aged black garlic [22,46] or red garlic [36]. This is reportedly attributable to melanoidins [35,36,46,49], which are the browning substance in aged heat-treated garlic. Also, black garlic is known to variable bioactivities such as antioxidant [45,46], improvement of cardiovascular complications of diabetes [42], decline of lipids accumulation in hypercholesterolemic rats [22]. It was demonstrated that S-allyl cysteine [10,20] and polyphenol compounds in garlic exert strong antioxidant effect [48]. Otherwise, antioxidant activities of red garlic − light color than black garlic −was more higher than the fresh garlic but was more lower than the black garlic, was reported that the antioxidant activity by

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production of the browning substance through the thermal process was the main parameter of the biological activity [36].

Despite such excellent bioactive function of black garlic compared to fresh garlic, the heat treatment of garlic has a down side such as high cost due to long times of its processing and limitations of 2^{nd} processing due to dark black color and high viscosity [36]. Therefore, this study aims to explore the anti-obesity and hypolipidemic effects by combining heat-treated red garlic extract in obese rats induced high fat diet, considering the fact that the red garlic developed through the aging process with low temperature showed higher bioactive effects than fresh garlic [36].

Materials and Methods

Preparation of extract

Red garlic used in this experiment were prepared manually, whole parts of fresh garlic were utilized for the manufacture of red garlic. The manufacturing protocol followed the patent application (No. 10-2010-0036798) by controlling of temperature and moisture. One hundred grams of red garlic removed peel was refluxed 2 times in 1000 ml water for 3 hr, at the 120℃. The hot-water extract was filtrated, concentrated under reduced pressure, and then fixed to 15 brix concentration. The extract was stored at 4℃.

Animals

Male Sprague-Dawley (SD) rats were purchased from Samtako Bio Korea Inc. (Osan, Korea). The anti-obesity and hypolipidemic studies were performed, aged 4 weeks and weighing 100±10 g for this experiment. All rats were housed individually in polypropylene cages and maintained under standard conditions of 22±2℃, relative humidity of 50±5% and a constant 12 hr (07:00-19:00) light and dark cycle. To help them adapt to the laboratory conditions, the rats were fed a standard pellet diet (SuperFeed Inc., Korea) with water *ad libitum* for the first week prior to the experiment.

For the experiments, the animals were randomly divided into 6 groups of 7 rats each. At the second week, rats were fed with a normal diet (12.40% of calories as fat) or high fat diet (40.54% of calories as fat). Control group fed high fat diet was not supplemented red garlic extract. Experimental group was divided to 4 groups by RG-1, -3, -5 and -7 group with supplemented red garlic extract of 1%, 3% 5% and 7% in total diet, respectively. All treatments continued for 4 weeks. Composition of the experimental diet is presented in Table 1.

Table 1. Composition of experimental diets (g/kg diet)

Diet composition	Normal1)	Control4)	Experimental groups5)			
			RG-1	RG-3	RG-5	RG-7
Casein	200	200	200	200	200	200
L-cystein	3	3	3	3	3	3
Dextrin	132	132	132	132	132	132
Corn starch	417.5	267.5	267.5	267.5	267.5	267.5
Sucrose	100	100	100	100	100	100
Soybean oil	50	50	50	50	50	50
Lard	0	150	150	150	150	150
Cellulose	50	50	50	50	50	50
Vitamin mixture2)	10	10	10	10	10	10
Mineral mixture3)	35	35	35	35	35	35
Choline bitartrate	2.5	2.5	2.5	2.5	2.5	2.5
Red garlic extract	-	-	10	30	50	70
Total energy (kcal)	3629.66	4439.66	4439.66	4439.66	4439.66	4439.66
Fat energy ratio (%)	12.40	40.54	40.54	40.54	40.54	40.54

1)Normal: Modified AIN-93G diet
2)AIN-93 vitamin mixture
3)AIN-93 mineral mixture
4)Control: High fat diet
5)RG-1: supplemented with 1% red garlic extract of 15 brix concentration, RG-3: supplemented with 3% red garlic extract, RG-5: supplemented with 5% red garlic extract, RG-7: supplemented with 7% red garlic extract.

Measuring of weight gains, food intakes and food efficiency ratio (FER)

During the experimental period, food intakes were monitored daily at the same time, while body weight was tested every week at same time. FER was calculated by total body weight gain (g)/total food intake amount (g) for 4 weeks.

Measuring of obesity index and fat content

To evaluate of obesity in rats, body weight and length from the tip of the nose to the anus in rats were measured at the last day for this experiment [26]. Obesity index such as Röhrer index, Lee index, T. M. index and body fat content was calculated using Röhrer index=[weight (g)/length $(cm)^3$]×10^3, Lee index=[weight $(g)^{1/3}$/ length (cm)]×10^3, T.M. index=weight (g)/length $(cm)^{2.823}$×10^3 and body fat content (%)=(0.581×T. M. index)-22.03.

Treatment of experimental rats

At the end of the experiment, after overnight fasting, all rats were anesthetized with diethyl ether. Blood samples from the heart were collected, cooled on ice and 30 min later the serum was separated by centrifuge (Mega 17R, HANILE, Korea) at 3000 rpm for 15 min at 4℃. Liver, heart, kidney, spleen and testis were excised rapidly, chilled and rinsed in ice-cold 0.9% NaCl, and weighed. The serum and organs of rat were stored under -70℃ before analysis.

Determination of lipids level in serum

Total lipid level was determined according to the method of Frings et al. [14]. 20 µl of serum was heated with 200 µl concentrated H_2SO_4 in a boiling water bath for 10 min, then 10 ml of phospho-vanillin solution was added, and the mixture was incubated 37℃ for 15 min, measured at 540 nm, and olive oil (0-500 mg) was used as standard for calibration curve. Total cholesterol, triglyceride and high density lipoprotein cholesterol (HDL-C) levels were determined with each kit reagent for total cholesterol (AM 202-k, Asan pharm. Co., Seoul, Korea), triglyceride (AM 157S-k, Asan, Korea) and HDL-C (AM 203-k, Asan, Korea). The low density lipoprotein cholesterol (LDL-C) and very low density lipoprotein cholesterol (VLDL-C) levels were calculated by the following formulas; LDL-C = TC－(HDL-C+TG/5) [13] and VLDL-C = TC－(HDL-C＋LDL-C) [9]. Atherogenic index (AI) was calculated by (TC－HDL-C)/HDL-C [17] and cardiac risk factor (CRF) was calculated by TC/HDL-C [23].

Determination of ALT and AST activities

To evaluate liver function, serum ALT (alanine aminotransferase) and AST (aspartate aminotransferase) activities were determined by commercial AM kit reagents (Asan, Korea) respectively.

Determination of lipid peroxide in serum

Serum (100 µl) was mixed 1/12 N H_2SO_4 solution and 10% phosphotungstic acid, then centrifuged at 4000 rpm for 10 min. The residue except the supernatant was mixed with distilled water and thiobarbituric acid (TBA) reagent. The mixture was incubated in a 95℃ water bath for 60 min and then cooled to room temperature in the ice bath. The mixture was added to exactly 4 ml *n*-butanol before being centrifuged at 3000 rpm for 10 min. The absorbance of the butanol fraction was measured at 532 nm. 1,1,3,3-tetraethoxypropan (TEP, Sigma Co., USA) was used as standard for calibration curve, its value was expressed as mmol/ml.

Antioxidant activity assay in serum

Antioxidant activity in the serum of rats was assayed according to the method of Lim et al. [38] with slight modifications. Serum (100 µl) was thoroughly mixed with 1 ml Tris-HCl buffer (100 mM, pH 7.4) and 2 ml of 1,1-diphenyl-2-picrylhydrazyl (DPPH, 2.5 mg/100 ml ethanol) was added rapidly. The mixture was incubated at 37℃ for 15 min before it was added to 4 ml chloroform. After centrifugation at 5000 rpm for 5 min, at 4℃, the absorbance of the chloroform layer was measured at 517 nm and chloroform was used as the blank. The antioxidant activity was expressed as the scavenging ratio of DPPH reagent color (%).

Determination of lipids level in tissues and feces

Lipids from the tissues were extracted according to the method of Folch et al. [12]. Tissue of 0.5 g or feces of 1 g were blended with the homogenizing stirrer (DAIHAN wise stir® HS-30E, Korea), and extracted well in a chloroform: methanol solution (2:1, v/v) at the room temperature for 24 hr, respectively. Three hundred µl each of the filtrate were exhausted by a rotary vacuum evaporator at 30-40℃ for determination of total lipids, total cholesterol and triglyceride content in tissues and feces. Determination of lipids level such as total lipid, total cholesterol and triglyceride in tissues and feces were evaluated using the same methods of lipids determination in serum, described above.

Statistical analysis

Values were presented as means±SD with n=7. Statistical difference between the treatments and controls were tested by one-way analysis of variance (ANOVA) followed by the Duncan's multiple range test. A difference in the mean values of p<0.05 was considered to be statistically significant. All statistical tests were performed using the computer software program SPSS for Windows (SPSS, version 12.0) for statistics and data analysis.

Results and Discussion

Weight change, FER and obesity index

The effects of the red garlic extract on the weight change, FER and obesity index in obese rats fed high fat diet are presented in Table 2. The control group fed high fat diet showed a weight gain of 186.75 g during the 4 weeks, which is about 1.2 fold higher than a normal group (157.75 g), whereas the experimental group fed with high fat diet added the red garlic extract did not show significant difference in weight change with compared to the control group. The significant difference was little depending on the amount of extracts. Food intake of the control and the red garlic extract groups was a little in significant difference with compared to the normal group, while FER was the highest in the control group fed high fat diet. Especially, the groups fed with 1-5% of red garlic extract had a higher than the control group, whereas the group fed with 7% of extract had a somewhat lower compared to the control group.

Obesity index using the body length and weight of rats were presented including Röhrer, Lee and T.M. index and body fat content. According to the experiment, the body length was similar to all experiment groups about 20.7 cm (data not shown), but the obesity index was significantly higher in the control group than that of the normal group. Meanwhile the obesity index in the experimental group was significantly decreased dose dependent of the added red garlic extract. Especially the groups fed with more than 5% of red garlic extract showed Röhrer index reduction to the level equal to the normal group. Lee and T.M index were significantly decreased in the groups fed over the 3% red garlic extract. Body fat content of the rats fed red garlic extract of 3% have declined with significant difference compared to the control group, except that there were no significant difference depending on the amount of added extract at the concentration of 3-7%.

In this study, we discovered that the high fat diet show higher FER in obese rats induced high fat diet even though it reduced the overall intake due to high fat content (40.54% of the total calorie) compared to that of the normal diet (12.40% of the total calorie). These result are similar to a reports that obese rats induced high fat diet takes in food at lower degree than a normal group, but the FER was higher [37,49], and which is reported to stem from the high calories by high fat [37]. Rats are considered obese when T.M. index is over 55 [30] or Röhrer index is over 30 [8]. Thus all the control and normal groups of this experiment were considered as an obese, though they are more or less obese. The evaluation by the obesity index results in difference outcomes depending on the lipids used for evaluation, and high fat diet-induced obese a somewhat higher obesity index than that is the case for cholesterol diet [8,26].

Weights of organs and fat in body

Table 3 is shown the effects of red garlic extract for the organs and fat weights in the obese rats induced high fat diet. The weights of a liver, heart and spleen of the control group has significantly increased compared to the normal

Table 2. Body weight gain, food intake, FER and obesity index in obese rats induced high fat diet

Groups[1)]	Total body weight gain (g/4 wk)	Food intake (g/day)	FER (%)	Röhrer index	Lee index	T.M. index	Body fat content (%)
Normal	157.75±7.23$^{a2,3)}$	20.00±0.09^{b}	28.84±1.81^{a}	31.37±2.12$^{5)a6)}$	309.29±6.86^{a}	53.98±3.44^{a}	9.36±0.70^{a}
Control	186.75±13.55^{b}	18.74±1.03^{a}	38.77±0.94^{bc}	37.85±0.99^{d}	329.39±2.85^{c}	64.31±1.67^{b}	15.34±0.97^{b}
RG-1	200.75±11.32^{b}	18.21±0.43^{a}	39.80±1.75^{c}	35.83±0.94^{cd}	323.45±2.58^{bc}	61.66±1.45^{b}	13.86±0.77^{b}
RG-3	192.00±25.10^{b}	18.61±0.71^{a}	41.06±1.72^{c}	34.58±1.95^{bc}	319.37±6.05^{b}	57.03±4.28^{a}	11.10±2.49^{a}
RG-5	198.25±24.07^{b}	19.19±0.58^{ab}	40.27±1.93^{c}	32.14±1.52^{a}	318.82±10.73^{b}	55.76±2.42^{a}	10.36±1.41^{a}
RG-7	179.75±17.17^{ab}	19.15±0.35^{ab}	37.03±2.13^{b}	32.97±0.74^{ab}	314.46±2.39^{ab}	56.85±1.08^{a}	11.00±0.63^{a}

[1)]See the legend of Table 1

[2)]Values are mean±SD (n=7).

[3)]Values in a column sharing the same superscript letter are not significantly different at p<0.05.

FER: food efficiency ratio.

Table 3. Effect of red garlic extract in the weight of organs, visceral and epididymal fat in obese rats induced high fat diet

Groups[1)]	Normal	Control	RG-1	RG-3	RG-5	RG-7
Tissues (g/100 g body weight)						
Liver	2.98±0.36[a2,3)]	3.56±0.21[c]	3.30±0.21[abc]	3.41±0.08[bc]	3.08±0.20[ab]	3.03±0.05[a]
Heart	0.31±0.02[b]	0.35±0.02[c]	0.30±0.01[b]	0.29±0.01[ab]	0.28±0.01[a]	0.31±0.01[b]
Kidney	0.61±0.05[NS]	0.66±0.02	0.64±0.03	0.61±0.03	0.62±0.04	0.66±0.07
Spleen	0.17±0.03[a]	0.21±0.01[b]	0.17±0.01[a]	0.18±0.02[ab]	0.17±0.02[a]	0.16±0.02[a]
Testis	0.95±0.06[bc]	0.98±0.06[c]	0.85±0.03[a]	0.88±0.06[ab]	0.91±0.03[abc]	0.90±0.08[abc]
Fat (g/100 g body weight)						
Visceral fat	0.94±0.51[NS]	1.41±0.27	1.18±0.41	1.16±0.14	1.12±0.11	1.15±0.14
Epididymal fat	1.48±0.38[a]	2.21±0.47[b]	1.96±0.37[ab]	2.09±0.24[b]	1.93±0.50[ab]	1.91±0.07[ab]

[1)]See the legend of Table 1

[2)]Values are mean±SD (*n*=7).

[3)]Values in a same row sharing the same superscript letter are not significantly different at $p<0.05$.

NS: Not significant.

group, whereas the weight of kidney and testis did not show any significant difference. The weight of liver tissue was significantly decreased in the group fed 5% of red garlic extract, while the weight of heart decreased for all groups fed with red garlic extract significantly to reach the level similar to the normal group. The weight of visceral fat did not show significant difference among all the experimental groups. But, the weight of epididymal fat in the control group was significantly increased compared to the normal group, whereas they showed no significant difference with groups fed red garlic extract.

In obese rats induced high fat diet, supplementation of *Allium victorialis* var. *platyphyllum* leaves in their diet showed decrease in the weight of total abdominal and epididymal fat compared to control group fed high fat diet [7]. In this study, however, feeding of the red garlic extract did not show a significant difference on the weight of internal body fat. But, feeding over 5% of red garlic extract was effective in the weight decline of liver and heart tissues compared to the control group.

Lipids level in serum

The serum lipids level of rats fed high fat diet added red garlic extract for 4 weeks were described in Table 4. Total lipid level of the control group was significantly higher than the normal group, and which decreased depending on the supplementation of red garlic extract in the experimental groups. The total cholesterol level did not show significant difference between the control group and the groups fed red garlic extract (RG-1－7). Triglyceride level of the control group was about 1.5 fold higher than the normal group, and which showed the decrease of 34.5-46.9% in those groups fed with red garlic extract. The HDL-C level of the control group fell compared to the normal group, though that of the experimental groups was recovered to similar level with the normal group. In terms of LDL-C level, there were no

Table 4. Effect of red garlic extract on lipid profiles in serum of the obese rats induced high fat diet

Groups[1)]	Normal	Control	RG-1	RG-3	RG-5	RG-7
Total lipid (mg/dl)	208.95±8.30[d2,3)]	267.72±6.87[e]	190.17±9.95[c]	176.42±9.76[b]	156.84±7.88[a]	146.99±9.28[a]
Total cholesterol (mg/dl)	53.03±3.52[a]	61.38±5.67[b]	59.12±2.09[ab]	59.75±5.79[ab]	59.51±2.66[ab]	58.19±6.26[ab]
Triglyceride (mg/dl)	43.81±2.09[c]	64.56±1.62[d]	42.30±5.74[bc]	38.84±2.55[ab]	38.07±2.35[ab]	34.31±2.73[a]
HDL-C (mg/dl)	31.57±0.63[bc]	28.17±0.98[a]	30.67±0.47[b]	30.27±1.42[b]	32.21±0.97[c]	32.21±0.72[c]
LDL-C (mg/dl)	14.46±3.14[a]	22.15±4.73[b]	21.01±1.49[ab]	19.82±4.40[ab]	18.87±2.38[ab]	16.84±3.66[ab]
VLDL-C (mg/dl)	8.76±0.42[c]	12.91±0.32[d]	8.46±1.15[bc]	7.77±0.51[ab]	7.61±0.47[ab]	6.86±0.55[a]
AI	0.68±0.14[a]	1.18±0.18[c]	0.93±0.08[b]	0.97±0.13[b]	0.85±0.08[ab]	0.81±0.18[ab]
CRF	1.68±0.14[a]	2.18±0.18[c]	1.93±0.08[b]	1.97±0.13[b]	1.85±0.08[ab]	1.81±0.18[ab]

[1)]See the legend of Table 1

[2)]Values are mean±SD (*n*=7).

[3)]Values in a column sharing the same superscript letter are not significantly different at $p<0.05$

HDL-C: High density lipoprotein cholesterol, LDL-C: Low density lipoprotein cholesterol, VLDL-C: Very low density lipoprotein cholesterol, AI: Atherogenic index, CRF: Cardiac risk factor.

significant difference between the control group and the group fed red garlic extract, whereas the VLDL-C level of groups fed red garlic extract decreased significantly compared to the control group and the ratio of drop was higher than that of LDL-C.

AI and CRF due to high fat diet were increased compared to the normal group, while tend to decrease by feeding of red garlic extract. Among the supplementation level of red garlic extract did not show significant difference, but in the groups fed 5-7% of red garlic extract, it reduced the AI by 28.0-31.4% and the CFR by 15.1-17.0%, compared to the control group.

Choi et al. [7] reported that feeding with *Allium victorialis* var. *platyphyllum* leaves in the rats induced high fat diet for 2 weeks revealed remarkable reduction of the total cholesterol, LDL-C and triglyceride in serum. The decline of HDL-C level and the increase of LDL-C level in serum are known as major contributors of arteriosclerosis [3,32]. According to Chi et al. [4], the elevation of cholesterol level in serum results from the increase of VLDL-C, and garlic is known to reduce the risk of arteriosclerosis by decreasing the VLDL-C in serum. In particular, the drop of triglyceride is due to the decomposition of chylomicrone, and VLDL-C is stimulated by the lipoprotein on the blood vessel walls [28]. Also, there is a report that AI was reduced due to the increased absorption of garlic, which in turn was possible by the generation of allithiamine when garlic was injected in combination with fermented soybean gemmule [6]. Ajoene, a type of allicin, of garlic decreased microviscosity of internal region within the lipid bilayer membrane of intact platelets as well as artificial lipid membrane, without affecting the outer hydrophilic properties of the bilayer [41]. Reportedly, effect of lipid improvement in garlic is due to an emulsion that is formed between lipid and components of garlic [1]. It is inferable that this applies to red garlic, as well.

ALT and AST activities

Serum ALT and AST activities in obese rats induced high fat diet presents in Table 5. It was significantly higher in the control group than the normal group. All experimental groups fed with red garlic extract showed significant decrease of ALT activity, and in the groups fed with 3-7% of red garlic extract, the AST activity significantly was decreased. The ALT activity rises in the liver disorder such as hepatitis, hepatic necrosis, and cirrhosis occur, wheras AST activity is known to be increasing in hepatitis, obstructive jaundice and myocardial infarction occur [43]. However ALT and AST activation tend to decreases according to the antioxidant activity of components added to diet of rats [40]. Lee et al. [36] noted that red garlic has the antioxidant activities higher than fresh garlic and similar to black garlic because it has a lot of polyphenol, flavonoids, thiosulfate and browning components more than fresh garlic. Thus it is regarded that ALT and AST activities in the serum of obese rats induced high fat diet is related to the above mentioned substance of the ingredients.

Table 5. Effect of red garlic extract on ALT and AST activities in serum of the obese rats induced high fat diet

Groups[1)]	ALT	AST
	(Karmen unit/ml)	
Normal	46.31±4.13[a]	148.21±2.87[a2,3)]
Control	56.98±0.94[b]	173.22±5.31[b]
RG-1	48.00±2.12[a]	169.29±9.00[b]
RG-3	44.36±5.26[a]	155.89±8.50[a]
RG-5	46.75±4.87[a]	154.46±6.27[a]
RG-7	43.34±4.43[a]	156.25±3.89[a]

1)See the legend of Table 1

2)Values are mean±SD (n=7).

3)Values in a column sharing the same superscript letter are not significantly different at p<0.05.

ALT: Alanine aminotransferase, AST: Aspartate aminotransferase.

Lipids level in liver and heart tissues

Lipids level in liver and heart tissues after 4 weeks feeding of the red garlic extract to obese rats induced high fat diet are shown in Table 6. The total lipid level in liver was 27.12 mg/g for the normal group and 37.06 mg/g for the control group, meaning that the control group has about 1.4 fold higher than the normal group. Its level showed decreasing trend disproportionately to the dose amount of red garlic extract and the decline was significant in the supplementation for 5-7% red garlic extract to the control group. The total cholesterol and triglyceride levels of the control group increased significantly compared to the normal group, but supplementation of red garlic extract with high fat diet did not show significant difference with compared to the control group. The total lipid level in heart tissue of the control group (25.82 mg/g) was about 1.3 fold higher than that of the normal group (19.38 mg/g). The groups fed with red garlic extract showed decreasing trend as the amount of extract increase and there was a significant decrease in the supplementation of red garlic extract for over 5%. The total cho-

Table 6. Effect of red garlic extract on lipid profiles in liver and heart tissues the obese rats induced high fat diet (mg/g, wet tissues)

Groups[1)]	In liver			In heart		
	Total lipid	Total cholesterol	Triglyceride	Total lipid	Total cholesterol	Triglyceride
Normal	27.12±1.31[a2,3)]	2.04±0.11[a]	2.04±0.11[a]	19.38±2.44[a]	1.34±0.21[a]	3.30±0.67[a]
Control	37.06±1.84[d]	2.55±0.15[b]	2.55±0.15[b]	25.82±1.96[c]	2.01±0.11[b]	7.94±0.27[b]
RG-1	35.80±3.56[cd]	2.45±0.43[b]	2.45±0.43[b]	24.57±2.37[bc]	1.57±0.51[a]	7.44±0.97[b]
RG-3	34.14±2.13[acd]	2.47±0.26[b]	2.47±0.26[b]	22.38±1.31[abc]	1.44±0.21[a]	7.01±0.13[b]
RG-5	33.19±2.06[ac]	2.41±0.24[b]	2.41±0.24[b]	20.91±3.14[a]	1.44±0.19[a]	6.82±0.92[b]
RG-7	32.11±1.47[a]	2.43±0.08[b]	2.43±0.08[b]	21.17±1.51[ab]	1.23±0.25[a]	6.86±1.25[b]

[1)]See the legend of Table 1
[2)]Values are mean±SD (n=7)
[3)]Values in a column sharing the same superscript letter are not significantly different at p<0.05.

lesterol level went down significantly in the groups fed red garlic extract compared to the control group, but there was no significant difference in the triglyceride level.

Yoon et al. [51] reported that supplementation of spices (red pepper, garlic and ginger) in the high fat diet showed more effective of the lipids improving for the fat tissue and serum in the garlic compared to pepper or ginger. The phenolic compounds in plant kingdom decreased lipid level in serum [48] and liver tissues [19], curtailing the occurrence of cardiovascular disease. Especially, it was reported that allicin is a major substance that decreases lipids level in the body [52]. Also, it was reported that the fresh garlic is heat-treated into red garlic and black garlic, the phenolic compounds level tend to elevate [36,45]. Therefore, the red garlic extract is believed to be improving the lipid profiles in the liver and heart tissues of obese rats.

Lipids level in feces

Table 7 presented the measurements of the lipids level of excrements depending on the period of red garlic extract feeding in obese rats induced high fat diet. The total lipid level of the control group was higher than that of the normal group after 1 week and 4 weeks, but in the groups fed red garlic extract, no significant difference as added amount of red garlic extract. Excrements of the total cholesterol recorded an increase when rats are fed with red garlic extract for 4 weeks, and the significant difference from the control group was appeared when the extract accounted for over 3%. Triglyceride level of the control group in the feces showed a significant increase compared to the normal group, while significantly higher increase was observed from the groups fed 5-7% red garlic extract than the control group.

Grape seed extract was reported to be effective in restricting absorption of dietary lipids in the body by increased excretion of triglyceride in obese rats induced high fat diet was about 1.2 fold with compared to the control group [5]. Lee [34] and Shimomura et al. [44] reported that even if rats take in large amount of fat, the amount of excrements remain consistent and thus lipid absorption rate of high fat diet group stands on a high level. However, this study revealed that supplementation of red garlic extract for obese rats restricts absorption of dietary lipids while increasing lipids excretion at the same time, resulting in the reduction lipids

Table 7. Lipid profiles in feces after 1 and 4 weeks' feeding of red garlic extract in the obese rats induced high fat diet (mg/g, dried feces)

Groups[1)]	Total lipid		Total cholesterol		Triglyceride	
	1 week	4 weeks	1 week	4 weeks	1 week	4 weeks
Normal	15.43±1.11[a2,3)]	15.85±0.37[a]	1.58±0.18[a]	1.61±0.09[a]	0.23±0.02[a]	0.24±0.02[a]
Control	29.75±2.23[b]	27.56±1.39[b]	2.57±0.08[b]	2.56±0.11[b]	0.61±0.06[a]	0.57±0.02[b]
RG-1	27.92±2.78[b]	27.78±3.09[b]	2.46±0.08[b]	2.44±0.26[b]	0.67±0.10[ab]	0.69±0.34[b]
RG-3	30.05±0.80[b]	29.85±3.08[b]	2.73±0.11[b]	3.70±0.71[c]	0.86±0.18[b]	0.87±0.13[bc]
RG-5	29.74±0.82[b]	29.25±0.54[b]	2.71±0.33[b]	3.78±0.59[c]	0.68±0.05[ab]	1.02±0.24[c]
RG-7	28.20±1.73[b]	30.02±1.08[b]	2.75±0.03[b]	3.54±0.06[c]	0.67±0.16[ab]	1.08±0.04[c]

[1)]See the legend of Table 1
[2)]Values are mean±SD (n=7).
[3)]Values in a column sharing the same superscript letter are not significantly different at p<0.05.

Table 8. Effect of red garlic extract on lipid peroxide level and antioxidant activity in serum of the obese rats induced high fat diet

Groups[1)]	Lipid peroxide (mmol/ml)	Antioxidant activity (%)
Normal	29.59±1.51[c2,3)]	57.29±0.93[c]
Control	40.95±1.89[d]	46.26±2.45[b]
RG-1	13.23±1.54[b]	38.60±5.48[a]
RG-3	12.00±2.96[b]	43.48±1.57[ab]
RG-5	8.05±1.14[a]	47.37±1.35[b]
RG-7	8.30±0.74[a]	52.51±2.85[c]

1)See the legend of Table 1
2)Values are mean±SD (*n*=7).
3)Values in a column sharing the same superscript letter are not significantly different at $p<0.05$.

level *in vivo*.

Lipid peroxidation and antioxidant activity in serum

Lipid peroxide level and antioxidant activity in serum were significantly higher in the control group compared with the normal group, as shown in Table 8. Lipid peroxide level was significantly decreased in the groups fed red garlic extract; such that the level in the groups fed 5-7% extract was lower compared to the groups fed 1-3% of red garlic extract. Lipid peroxide level in serum increased with high fat diet consumption [15], high fat diet was more prone to oxidation and generation of free radicals which was positively correlated with total cholesterol and triglyceride in serum [39].

Antioxidant activity by DPPH radical scavenging in serum was significantly increased in the group fed 7% red garlic extract compared to the control group. Its activity was similar to the normal group. Jastrzebski et al. [21] reported plasma antioxidant activity in the rats fed cholesterol was found a significant increase in the groups added the fresh and heat-treated garlic for 20 min. It is known that phenolic compounds in the plants have antioxidant activity [33] by obstructing generation of malondialdehyde [2]. We suggest that the results of this experiment involved interaction of phenolic and browning compounds of red garlic by heat treatment. And dietary hypolipidemic garlic was effective in reducing the oxidative stress, which was indicated by an increase of antioxidant activity and a decrease of lipids level in the rats.

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초록 : 홍마늘 추출물이 고지방식이로 유도된 비만 흰쥐의 항비만 및 지질개선에 미치는 영향

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고지방식이로 유도된 비만흰쥐에 15 brix의 홍마늘 열수추출물을 1%, 3%, 5% 및 7%로 식이 중에 혼합급이하여 체내 지질 함량 및 항비만 효과를 측정하였다. 비만지수와 체지방량은 3% 이상의 홍마늘 추출물의 급이시 대조군에 비해 유의적으로 감소되었으나, 내장 지방 및 부고환 주변 지방 함량은 홍마늘 추출물의 급이에 따른 유의차가 없었다. 혈청 중의 총 지질 및 중성지방은 추출물의 첨가량이 증가됨에 따라 유의적으로 감소하였으며, 동맥경화지수와 심혈관질환 위험지수도 감소되었다. ALT 및 AST 활성은 홍마늘 추출물 급이시 대조군에 비해 감소되었다. 간 조직 중 총 지질 함량은 5-7%의 홍마늘 추출물 급이군에서 유의적으로 감소되었으며, 홍마늘 추출물의 5% 이상 급이시 분변 중에 총 콜레스테롤 및 중성지방의 배설이 증가되었다. 혈청 중 지질 과산화물 함량은 5-7%의 홍마늘 추출물 급이군에서 유의적으로 감소되었으며, 항산화 활성은 7% 급이군에서 유의적으로 상승되었다. 따라서 홍마늘 추출물은 분변으로 콜레스테롤 및 중성지방의 배출을 증가시킴으로써 체내 지질 저하 및 항비만 효과를 내는 것으로 사료된다.