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Hypolipidaemic Effect of Processed Sulfur, *Allium tuberosum* Rottl. and Fermented *Allium tuberosum* Rottl. in Rat

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ABSTRACT

This study was performed to determine the hypolipidaemic effects of *Allium tuberosum* Rottl. (ATR), fermented *Allium tuberosum* Rottl. (FATR) and processed sulfur. Thirty male Wistar rats were divided into five groups: (i) CON (control, normal diet); (ii) HFD (high fat diet); (iii) HS (HFD+processed sulfur); (iv) AS (HFD+ATR+processed sulfur) and (v) FAS (HFD+FATR+ processed sulfur). Animals were fed the diets for six weeks. Body weights of the animals were recorded in the beginning and end of experiment. The animals were sacrificed, organ and tissue weights recorded and blood and liver samples were analyzed for various biochemical parameters. Body weight gain in the AS group was significantly ($p<0.05$) lower than the other groups. Spleen and kidney weights in FAS group were significantly ($p<0.05$) higher than the other groups. The weight of epididymal adipose tissue in AS group was significantly ($p<0.05$) lower than that of HFD group. The weight of retroperitoneal fat in HFD groups was significantly ($p<0.05$) higher than other groups. The concentration of aspartate amino transferase (AST) was significantly ($p<0.05$) higher in HFD group compared to HS, AS and FAS groups. Total liver lipid contents in the HFD group was significantly ($p<0.05$) higher than the other groups. AS and FAS group rats had significantly ($p<0.05$) lower triglyceride and total cholesterol compared to HFD group. All together, these results suggest that FATR and processed sulfur may play an important role in reducing body fat deposition and altering the serum and liver lipid profile in rats.

Key words: *Allium tuberosum* Rottl., processed sulfur, body fat deposition, serum lipid profile, liver lipid profile

INTRODUCTION

General fatness of animals increases with intake of excess calorie as it is converted into fat and accumulate in various parts of the interior of the body in the form of hypodermic or intra abdominal fats. Excessive accumulation of fat has adverse effects on physiological and biochemical functions of the body (Ryu *et al*., 2006). The WHO (World Health Organization) and other international authorities have recognized that diseases such as arthritis and cardio vascular disorder are increasing globally due to excessive fatness (Lee *et al.*, 2008; Jang and Jeong, 2010). Recent developments in functional food ingredients isolated from natural substances such as flavonoid from plants, chitosan from shell fishes and catechin of green tea have shown great promise in reducing fatness (Zacour *et al.*, 1992). Hung *et al.* (2008) reported hypolipidaemic effect of fermented soy meal with low serum cholesterol and triglyceride in pigs.

Allium tuberosum Rottl. (ATR) is a plant in the *Allium* group, and from old time it is being used habitually as herbs for its unique scent and taste. It is also reported to be effective in dismantling of fat cell (Hwang *et al.*, 2001). Sulfur-containing compounds present in garlic, onions and ATR of the Allium group are reported to have various physiologic effects including antioxidation, detoxification and cardiovascular protection (Kim *et al.*, 2005; Park *et al.*, 1998; Pinto *et al.*, 1997; Senapati *et al.*, 2001; Lee *et al.*, 2002). Onion (*Allium cepa*) is reported to have hypoglycemic, hypolipidaemic and cardioprotective effect and its potential use in diabetes and cardiovascular diseases is suggested (Sohail *et al.*, 2011). Allicin, an organosulfur compound derived from *Allium sativum* has been reported to have hepatoprotective effects in rats (Attia and Ali, 2006). Abdel Gadir *et al.* (2006) has reported the dietary effect of *Allium cepa* and *Allium sativum* in rats. Abdel Gadir *et al.* (2007) has reported that *Allium cepa*, *Allium sativum* or sodium selenite in combination with potassium bromate has no adverse effect in Wistar rats. ATR have high concentration of chlorophyll, dietary fiber and adenosine, an antithrombotic component which have effects to decrease serum cholesterol and triglyceride (Hong and Wang, 2000). It is also reported to have anticarcinogenic effects on human cancer cells (Park *et al.*, 2002).

After removal of toxicity through processing, sulfur has been prescribed for homeostasis, paralysis and known to prevent osteoporosis and experimental bone disease (Chae *et al.*, 2007; Kim and Seo, 1996). Sulfur-containing ducks are currently raised in domestic production facilities to get the benefit of sulfur containing diet.

This study was carried out to determine the effects of *Allium tuberosum* Rottl. (ATR), fermented *Allium tuberosum* Rottl. (FATR) and sulfur added to a high fat diet on growth, body fat deposition, organ weights, serum and liver biochemistry and lipid profile in rats.

MATERIALS AND METHODS

This study was carried out during the period from February 2010-February 2011.

Preparation of ATR and FATR: *Allium tuberosum* Rottl. (ATR) was purchased from the local market (Seoul, Korea) and processed sulfur was received from Ebada Eco Technology (Seoul, Korea). The tubers of ATR were cleaned, de-skinned and then ground in a blender (Angel-juicer, Busan, Korea) and filtered through gauze (Daegu, Korea). This filtrate was used as ATR extract. For preparation of fermented *Allium tuberosum* Rottl. (FATR), the filtrate was added to de Man, Rogosa and Sharpe (MRS) medium inoculated with *Leuconostoc mesenteroides* at 1:1(v/v) ratio and incubated at 30°C for 16 h. ATR and FATR extracts were freeze-dried (Ilshin Lab. MCFD 8510, Suwon, Korea) and the powder was used in the experimental diets.

Analysis of processed sulfur, ATR and FATR: The processed sulfur content of freeze-dried ATR and FATR was measured without preprocessing using an EA 1110 (ThermoQuest CE Instruments, Italia) analysis instrument.

The moisture, ash, crude protein, crude fat, crude fiber and carbohydrates percentage in ATR and FATR powder were estimated according to Association of Official Analytical Chemists

Table 1: Composition of experimental diets (g kg^{-1} diet)

	Groups				
Ingredient	CON	HFD	HS	AS	FAS
Casein	200	233.1	233.1	209.6	209.6
Sucrose	100	201.4	201.4	181.1	181.1
Dextrose	132	116.5	116.5	104.8	104.8
Corn starch	398	84.8	84.8	76.3	76.3
Cellulose	50	58.3	56.8	52.4	52.4
Soybean oil	70	29.1	29.1	26.2	26.2
Lard	0	206.9	206.9	186	186
Processed sulfur	-	0.0	1.5	0.870	0.870
ATR[1]	-	0.0	0.0	100.0	0.0
FATR[2]	-	0.0	0.0	0.0	100
Mineral mix	35	52.4	52.4	47.2	47.2
Vitamin mix	10	11.7	11.7	10.5	10.5
L-cystine	3	3.5	3.5	3.1	3.1
Choline bitartrate	2.5	2.33	2.33	2.1	2.1
Total (g)	1000	1000	1000	1000	1000
Total (kcal)	4000	4661	4661	4661	4661

[1]ATR: *Allium tuberosum* Rottl., [2]FATR: Fermented *Allium tuberosum* Rottl. CON: Control diet, HFD: High fat diet, HS: High fat diet+processed sulfur, AS: High fat diet+*Allium tuberosum* Rottl.+ processed sulfur, FAS: High fat diet+fermented *Allium tuberosum* Rottl.+processed sulfur

(AOAC, 2005) methods. Moisture (%) was determined using the drying method at 105°C; ash content was estimated by dry ashing at 550°C; crude protein was determined by the micro-Kjeldahl method; crude fat by the Soxhlet method and crude fiber was determined using a fiber extractor (Fibretherm, Gerhardt, USA). Carbohydrate content was calculated by subtracting the sum total of moisture, ash, crude protein, crude fat, crude fiber contents (%) from 100 (carbohydrate = 100-(moisture+protein+fat+ash).

Experimental animals and diets: A total of 30 male, five-weeks-old Wistar rats, obtained from Central Laboratory Animal Inc., Seoul, Korea, were acclimatized for one week. Then the animals were randomly assigned to one of five diet groups (n = 6):(i) CON (control, normal diet);(ii) HFD (high fat diet, AIN-93G diet); (iii) HS (HFD+processed sulfur); (iv) AS (HFD+ATR+processed sulfur) and (v) FAS (HFD+FATR+processed sulfur). The rats were fed a restricted diet (20 g) and allowed free access to tap water. The animal room was maintained at controlled temperature (22±2°C) and humidity (50±5%) with a 12 h light-dark cycle. The experiment continued for 6 weeks. The sulfur content of the feed was 30 mg/day/rat (Lee *et al*., 2008). The other ingredients of the experimental diets are detailed in Table 1.

Parameter analysis: Body weight was recorded weekly using an electronic scale (Adventurer AR2140; OHAUS Corp., USA). Food intake (g) was calculated by subtracting the amount (g) of food left in the next day from the amount (g) of food supplied in the previous day. After the experimental feeding period was over the animals were sacrificed and the spleen, liver, kidney, epididymal adipose tissue and retroperitoneal fat were removed and weighed. Liver samples were stored at -80°C until analyzed.

Blood was collected from a postcaval vein and centrifuged at 3,000 rpm for 15 min. The serum was frozen at -20°C until analyzed for Total Cholesterol (TC), Triglyceride (TG), High-Density Lipoprotein Cholesterol (HDLC), Low-Density Lipoprotein Cholesterol (LDLC), Atherogenic Index (AI), aspartate aminotransferase (AST), alanine aminotransferase (ALT) and glucose. AI was calculated as follows: [TC-HDLC]/HDLC (Haglund *et al.*, 1991). All assays were performed by Biotoxtech Co.

Total liver lipids were extracted as per (Folch *et al.*, 1957). A 50 mg sample of liver and 3 mL of distilled water were homogenized (HS-30E; Daihan Sci., Korea) and 7 mL of chloroform added. After centrifugation, the supernatant was removed and incrassated using Nitrogen (N_2) gas to measure total lipid weight.

To measure total cholesterol in the liver, samples were incrassated using N_2 gas, dissolved with 2 mL of ethanol and processed using a reagent kit (Asan Pharmaceutical Co., Korea). Enzyme reagent (3 mL) was added to liver samples (20 µL) and allowed to stand for 5 min at 37°C after which absorbance was measured at 500 nm.

For liver triglyceride analysis, dissolved samples (500 µL) and ethanol (2 mL) were mixed in Eppendorf tubes and the ethanol was then removed using N_2 gas and a solution (50 µL) containing triton X-100 and chloroform (1:1) were added and mixed with chloroform (450 µL). Enzyme reagent (3 mL) (Asan Pharmaceutical) was added and absorbance was measured at 550 nm after keeping at 37°C for 15 min.

Statistical analyses: The data were analyzed using the SAS (Statistic Analytical Institute, Cary, NC, USA) program. Significance was set at $p<0.05$ and Tukey's multiple range test (Snedecor and Cochran, 1989) was used for 95% significant difference comparisons.

RESULTS AND DISCUSSION

This experiment was conducted to find the effects of *Allium tuberosum* Rottl. *(ATR),* fermented *Allium tuberosum* Rottl. (FATR) and sulfur added to a high fat diet on growth, body fat deposition, organ weights, serum and liver biochemistry and lipid profile in rats.

Sulfur content (0.6±0.02%) of ATR was significantly ($p<0.05$) higher than that of FATR (0.4±0.01%). The proximate composition of ATR and FATR powder including crude fiber are shown in Table 2. Crude protein and crude fat percentage in ATR were significantly ($p<0.05$) higher than those of FATR, whereas, moisture and carbohydrate contents showed reverse trend.

The body weight and feed intake results for six weeks are depicted in Table 3. Initial body weight of all rats was approximately 181 g. Body weight gain in AS and CON group of rats was significantly ($p<0.05$) lower than that of FAS, HFD and HS group rats after 6 weeks, although feed intake (g) was similar for all the groups. Similar findings were reported by Jang and Jeong (2010) recording higher body weight gain in mice fed with high fat diet compared to mice fed with normal diet and diet containing phyto-extract mixture.

The results related to epididymal adipose tissue, retroperitoneal fat and some internal organ weights (g) are presented in Table 4 and Fig. 1a and b. Liver weights did not vary among the groups. FAS group rats had significantly ($p<0.05$) higher kidney weight compared to rats of other groups. Similarly the rats belonging to FAS group had significantly ($p<0.05$) higher spleen weight than other groups. Rats of HFD group deposited significantly ($p<0.05$) higher amount of retroperitoneal and epididymal fats compared to all other groups except HS and FAS group in

Table 2: The proximate composition (%) of ATR and FATR powder

	Groups	
Parameter	ATR	FATR
Moisture	0.1±0.02[b]	0.4±0.06[a]
Ash	4.2±0.12	4.4±0.14
Crude protein	35.9±0.72[a]	34.5±0.10[b]
Crude fat	0.7±0.08[a]	0.5±0.00[b]
Crude fiber	0.1±0.03[ND]	0.1±0.02
Carbohydrates	58.9±0.72[b]	60.2±0.09[a]

Means with different superscripts differ significantly ($p<0.05$), All values are mean ± SD of triplicate determinations

Table 3: Body weight gain and food intake (g) of rats fed CON, HFD, HS, AS and FAS diets for six weeks

	Body weight (g)			
Groups	Initial	Final	Body	Total food intake (g)
CON	181.2±5.88	345.5±7.29[bc]	164.3±1.41[b]	20.8±0.53
HFD	181.3±6.47	367.0±8.83[a]	185.7±2.36[a]	19.6±0.53
HS	181.0±5.69	361.3±6.25[ab]	180.3±0.56[a]	19.0±0.57
AS	181.0±5.55	342.5±11.83[c]	161.5±6.28[b]	19.7±0.37
FAS	181.3±6.44	369.5±14.01[a]	188.2±7.57[a]	20.0±0.35

Value which have different letters are significantly different ($p<0.05$), All values are Mean±SD (n = 5)

Table 4: Organ weights (g) of rats fed CON, HFD, HS, AS and FAS diets for six weeks

	Organ weight (g)		
Groups	Liver	Kidney	Spleen
CON	8.4±0.52	2.0±0.08[b]	0.5±0.03[bc]
HFD	8.8±0.74	2.0±0.07[b]	0.6±0.06[bc]
HS	8.8±0.45	2.1±0.10[b]	0.5±0.05[c]
AS	8.4±0.58	2.1±0.07[b]	0.6±0.02[ab]
FAS	9.2±0.17	2.3±0.13[a]	0.7±0.05[a]

Value which have different letters are significantly different ($p<0.05$), All values are mean±SD (n = 5)

respect of epididymal fat only. Ryu *et al.* (2006) reported that no difference in abdominal fat, paratesticular fat and perirenal fat weights of mice fed with HFD+Misaengtang (a Korean herbal formula) compared to HFD only. However, in the present study results revealed that ATR, FATR and processed sulfur had better anti-obesity effect than the traditional Korean herbal formula resulting in lower fat deposition in the experimental mice. Results on liver weights were similar to those reported by Ryu *et al.* (2006) but the present findings indicated positive effect of FAS on spleen and kidney weights.

Table 5 and 6 depict the results of enzyme and lipid profiles of rats belonging to different experimental groups. There was no effect on ALT in any group; however, AST in all groups (HS, AS, FAS) was significantly ($p<0.05$) lower than HFD group and lowest in the FAS group. HS group rats had significantly ($p<0.05$) lower blood glucose level compared to rats of AS group, however, rats of other groups did not show any significant difference in blood glucose level. Most importantly atherogenic index (AI) was lowest (2.3) in FAS group rats followed by AS (2.5), HS (2.7), CON (3.1) and HFD (3.3) group rats, although differences were statistically non-significant. Similar to the present findings, Ryu *et al.* (2006) reported that there was no effect of diets (HFD and

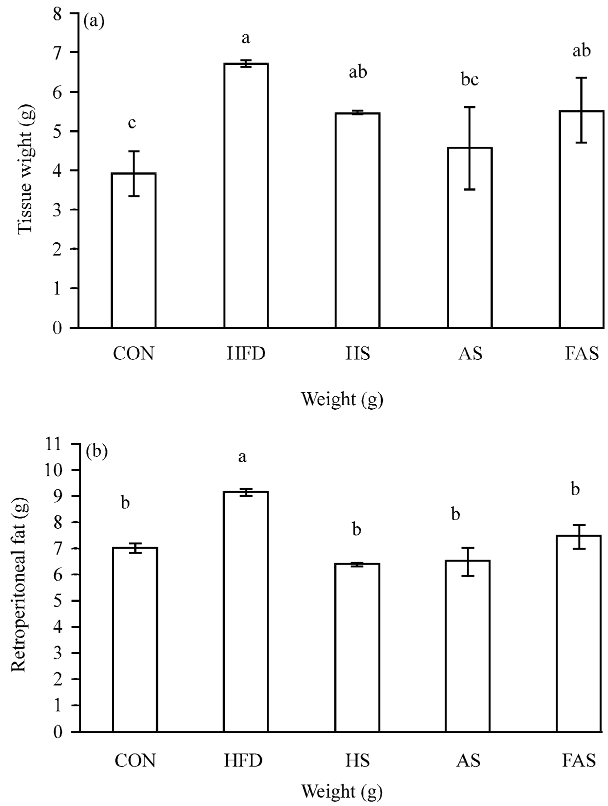

Fig. 1(a-b): (a) Epidermal adipose tissue and (b)Retroperitoneal fat weights (g) of rats fed CON, HFD, HS, AS and FAS diets for six weeks, Values which have different letters are significantly different ($p<0.05$), All values are Mean±SD (n = 5)

Table 5: Serum ALT, AST and glucose levels of rats fed with CON, HFD, HS, AS and FAS diets for six weeks

	Items			
Groups	ALT (U T^{-1})	AST (U T^{-1})	AI (atherogenic index)	Glucose (mg dL^{-1})
CON	55.3±6.00	187.4±11.17^{ab}	3.1±0.21	107.4±6.23^{ab}
HFD	69.0±6.10	201.0±7.84^{a}	3.3±0.16	116.6±13.87^{ab}
HS	60.5±5.60	167.7±17.16^{bc}	2.7±0.33	110.0±1.83^{b}
AS	69.0±2.10	146.6±7.29^{cd}	2.5±0.47	134.5±17.60^{a}
FAS	48.2±4.26	129.6±16.84^{d}	2.3±0.45	134.5±17.60^{ab}

Values which have different letters are significantly different ($p<0.05$), All values are Mean±SD (n = 5)

HFD+Korean herbal formula) at different levels on ALT and glucose concentration but significant effect of diet containing herbal formula on AST was reported by the same authors. Choudhary (2008) recorded significant positive effects of *A. sativum* and *A. tuberosum* on atherogenic index in guinea pigs.

Serum LDL-C level in AS and FAS group rats were significantly ($p<0.05$) lower than in the control group. But levels did not vary significantly among various treatment groups, although FAS group rats had lowest LDL-C level followed by AS, HFD and HS groups, respectively.

Serum HDL-C levels were significantly ($p<0.05$) lower in FAS and AS group rats compared to CON and HFD group rats. On the contrary, serum Total Cholesterol (TC) levels were significantly ($p<0.05$) higher in FAS group rats than in rats belonging to other groups. In case of serum TG rats fed with CON diet exhibited significantly ($p<0.05$) lower level compared to rats fed with HS, AS and FAS diets.

Choudhary (2008) reported significant reduction in cholesterol, triglycerides, LDL-C levels and AI in serum samples of guinea pigs fed with diet containing *A. sativum* and *A. tuberosum* but no effect was seen on HDL-C level. On the other hand Ryu *et al.* (2006) did not find any significant change in the serum levels of TC, HDL, LDL and TG in mice fed with diet of HFD+Misaengtang, a Korean herbal preparation.

Concentration of total lipid in liver of rats belonging to different groups is shown in Fig. 2. Total lipid content was significantly ($p<0.05$) higher in HFD group rats than that in other groups including control. AS group rats showed significantly ($p<0.05$) lower total lipid concentration in liver than in CON, HFD and HS group rats.

The results of adding sulfur, ATR and FATR to a high fat diet on total cholesterol are presented in Fig. 3. Total cholesterol in the AS and FAS groups were significantly ($p<0.05$) lower than in the HFD group. Liver triglyceride values are depicted in Fig. 4. Triglyceride concentration in liver of

Table 6: Serum levels of TC, TG, HDL-C, LDL-C, and AI of rats fed with CON, HFD, HS, AS, and FAS diets for six weeks

	Levels				
Groups	T-C (mg dL^{-1})	TG (mg dL^{-1})	HDL-C (mg dL^{-1})	LDL-C (mg dL^{-1})	AI
CON	58.4±4.20^{b}	41.3±2.87^{c}	17.5±1.23^{c}	3.8±0.17^{a}	2.34
HFD	66.4±5.03^{b}	58.4±10.41^{bc}	19.8±1.05^{c}	3.3±0.25^{ab}	2.36
HS	65.40±5.50^{b}	79.0±14.02^{a}	20.0±1.58^{bc}	3.4±0.21^{ab}	2.27
AS	64.5±4.80^{b}	61.3±4.04^{ab}	22.8±1.81^{ab}	3.0±0.64^{b}	1.83
FAS	76.5±3.42^{a}	68.2±5.87^{ab}	24.7±1.61^{a}	2.7±0.45^{b}	2.10

Values which have different letters are significantly different ($p<0.05$), All values are Mean±SD (n = 5)

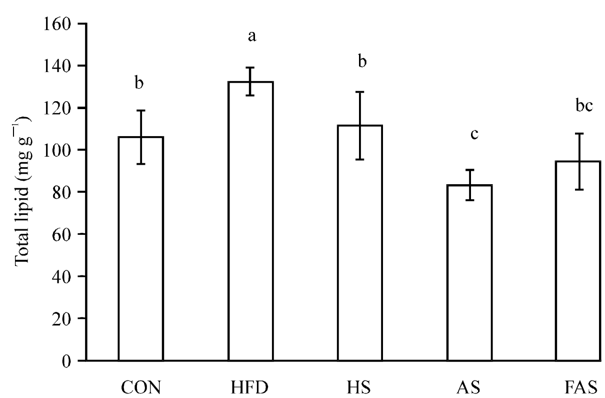

Fig. 2: Total lipid levels in the livers of rats fed with CON, HFD, HS, AS and FAS diets for six weeks. Values which have different letters are significantly different ($p<0.05$), All values are Mean±SD (n = 5)

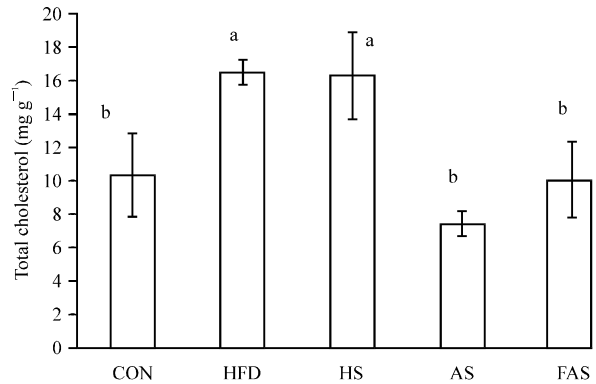

Fig. 3: Total cholesterol levels in the livers of rats fed with CON, HFD, HS, AS and FAS diets for six weeks, Values which have different letters are significantly different ($p<0.05$), All values are Mean±SD (n = 5)

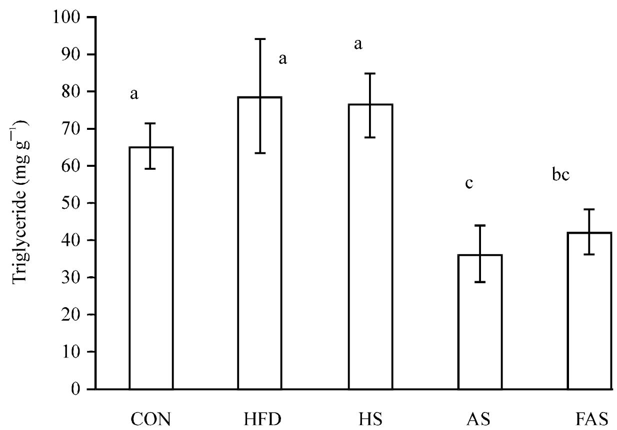

Fig. 4: Triglyceride levels in the livers of rats fed CON, HFD, HS, AS and FAS diets for six weeks. Values which have different letters are significantly different ($p<0.05$), All values are Mean±SD (n = 5)

rats belonging to AS and FAS groups was significantly ($p<0.05$) lower than that in rats of CON, HFD and HS groups.

CONCLUSION

This study was conducted to determine the effects of feeding *Allium tuberosum* Rottl. (ATR), fermented *Allium tuberosum* Rottl. (FATR) and processed sulfur added to a high fat diet for six weeks on body fat accumulation, blood and liver lipid profiles, body weight, organ weights, blood and liver biochemical parameters. Based upon the results, it can be concluded that rats experienced improvement in liver function and a reduction in body weight, intra-abdominal fat and serum LDL-C by consuming a high fat diet with the addition of FATR and processed sulfur. This study provides preliminary data regarding the effects of utilizing processed sulfur and ATR as food additives.

REFERENCES

AOAC, 2005. Official Methods Analysis. 18th Edn., Association of Official Analytical Chemists, Washington DC USA, pp: 125-132.

Abdel Gadir, E.H., W.S. Abdel Gadir and E.S.I. Adam, 2006. Response of Wister rats to low levels of dietary *Allium cepa, Allium sativum* Sodium selenite. J. Pharmacol. Toxicol., 1: 284-288.

Abdel Gadir, E.H., W.S. AbdelGadir and S.E.I. Adam, 2007. Toxicologic interaction of potassium bromate and *Allium cepa, Allium sativum* or sodium selenite in Wistar rats. J. Pharmacol. Toxicol., 2: 496-501.

Attia, M.N.T. and M.A. Ali, 2006. Hepatoprotective activity of allicin against carbon tetrachloride induced hepatic injury in rats. J. Boil. Sci., 6: 457-468.

Chae, M.H., J.G. No and D.Y. Jhon, 2007. Hangbisan, sulfur-based oriental medicine, lower the blood cholesterol level of ob/ob obese mice. J. Korean Soc. Food Sci. Nutr., 36: 27-31.

Choudhary, R., 2008. Beneficial effects of allium sativum and *Allium tuberosum* on experimental hyperlipidemia and atherosclerosis. Pak. J. Physiol., 4: 7-9.

Folch, J., M. Lees and G.H.S. Stanley, 1957. A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem., 226: 497-509.

Haglund, O., R. Luostarinen, R. Wallin, L. Wibell and T. Saldeen, 1991. The Effects of fish oil on triglycerides cholesterol fibrinogen and malondialdehyde in humans supplemented with Vitamin E. J. Nutr., 121: 165-169.

Hong, S.A. and S.G. Wang, 2000. Effects of Korean leek and dietary fat on plasma lipids and platelet aggregation in hypercholesterolemic rats. J. Korean Nutr. Soc., 33: 374-385.

Hung, A.T.Y., T.M. Su, C.W. Liao and J.J. Lu, 2008. Effect of probiotic combination fermented soybean meal on growth performance, lipid metabolism and immunological response of growing-finishing pigs. Asian J. Anim. Vet. Adv., 3: 431-436.

Hwang, C.W., H.K. Shin, M.S. Do, Y.J. Kim, J.H. Park, Y.S. Choi and W.H. Joo, 2001. The various biofunctional effects (anticarcinogenic, antioxidative and lypolytic activity) of Pohang Buchu. Korean J. Food Sci. Technol., 33: 279-281.

Jang, Y.S. and J.M. Jeong, 2010. Effects of phyto-extract mixture on adiposity and serum lipid levels in obese mice induced by high fat diet. J. Korean Soc. Food Sci. Nutr., 39: 1439-1445.

Kim, J.H., H.Y. Seo, K.M. No, B.J. Han, S.J. Lee, Y.S. Seo and K.S. Kim, 2005. Changes of volatile odor components in onion by freeze-drying. J. Korean Soc. Food Sci. Nutr., 34: 230-235.

Kim, S.H. and Y.B. Seo, 1996. Effect of processed sulfur on experimental bone disease. Korean J. Oriental Med. Pathol., 10: 79-87.

Lee, M.J., B.M. Ryu, Y.S. Lee and G.S. Moon, 2002. Effects of long term Buchu (Chinese chives) diet on antioxidative system of ICR mice. J. Korean Soc. Food Sci. Nutr., 31: 834-839.

Lee, S.K., S.H. So, E.I. Hwang, B.S. Koo, G.G. Han, S.B. Ko and N.M. Kim, 2008. Effects of ginseng and herbal plant mixtures on anti-obesity in obese SD rat induced by high fat diet. J. Korean Soc. Food Sci. Nutr., 37: 437-444.

Park, E.R., J.O. Jo, S.M. Kim, M.Y. Lee and K.S. Kim, 1998. Volatile flavor components of leek (*Allium tuberosum* Rottler). J. Korean Soc. Food Sci. Nutr., 27: 563-567.

Park, Y.J., M.H. Kim and S.J. Bae, 2002. Anticarcinogenic effects of allium tuberosum on human cancer cells. Korean J. Food Sci. Technol., 34: 688-693.

Pinto J.T., C. Qiao, J. Xing, R.S. Rivlin and M.L. Protomasto *et al.*, 1997. Effects of garlic thioallyl derivatives on growth, glutathione concentration, and polyamine formation of human prostate carcinoma cells in culture. Am. J. Clin. Nutr., 66: 398-405.

Ryu, J.M., T.H. Lee, I.K. Seo, S.H. Lee, Y.H. Chang, Y.B. Kim and S.Y. Hwang, 2006. Anti-obesity effects of Misaengtang in rats fed on a high-fat diet or normal diet. J. Toxicol. Publ. Health., 22: 339-348.

Senapati, S.K., S. Dey, S.K. Dwivedi and D. Swarup, 2001. Effects of garlic (*Allium sativum* L.) extract on tissue lead level in rats. J. Ethnopharmacol., 76: 229-232.

Snedecor, G.W. and W.G. Cochran, 1989. Statistical Methods. 8th Edn., Oxford and IBH Publishing Co., Calcutta.

Sohail, M.N., A. Karim, M. Sarwar and A.M. Alhasin, 2011. Onion (*Allium cepa* L.): an alternative medicine for Pakistani population. Int. J. Pharmacol., 7: 736-744.

Zacour, A.C., M.E. Silva, P.R. Cecon, E.A. Bambirra and E.C. Vieira, 1992. Effects of dietary chitin on cholesterol absorption and metabolism in rats. J. Nutr. Sci. Vitaminol., 38: 609-613.