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EFFECT OF FEEDING ON BEEF PATTIES FORTIFITED WITH MUSHROOM ON SERUM LIPIDS PROFILE AND LIVER ENZYMES OF HYPERCHOLESTEROLEMIC RATS

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ABSTRACT

The investigation was carried out to study the effects of feeding hypercholesterolemic rats on 35% beef patties fortified with 0, 4, 8 and 12% oyster dried mushroom on blood serum lipids profile and liver enzymes comparing with feeding on diets containing 8% dried mushroom and 8% defatted soy flour. The results showed that all diets had hypercholesterolemic lowering effect after 30 days comparing to 15 days. Moreover, group fed on 35 % beef patty fortified with 12% dried mushroom had the highest reduction of total cholesterol, triglycerides, LDL, VLDL, total lipids and phospholipids but, it had the highest increasing in HDL comparing to other groups. So, this group had the lowest atherogenic index as CH/HDL-c and LDL/HDL-c comparing to other groups. With respect to liver enzymes GOT and GPT, group fed on 35 % beef patties fortified with 12% dried mushroom had the highest lowering effect comparing to other groups. These results indicated that mushroom had the highest lowering hypercholesterolemic effect. Moreover, the group which fed on 8% defatted soy flour had the same hypercholesterolemic lowering effect of the group which fed on 8% dried mushroom on blood serum lipids profile and liver enzymes. The hypercholesterolemic lowering effect was increased by increasing fortification with dried mushroom in beef patties preparation and also, was increased by increasing feeding time.

Keywords: beef patties, dried mushroom, defatted soy flour, blood serum lipids, hypercholesterolemic, atherogenic index , liver enzymes.

INTRODUCTION

Human nutrition has become more complicated since the outbreak of diseases connected with animal meat. The human race has growing need for protein, so there is a reason for investigations of natural, especially vegetables and unconventional products, created as nutritional substitutes for traditional fast food (Krbavcic and Baric, 2004).

Mushrooms have been used in folk medicine throughout the world since ancient times. Many medicinal properties have been attributed to mushrooms, reduction of blood cholesterol levels, prevention or alleviation of heart diseases and reduction of blood glucose levels. Mushrooms have also been reported to block induced liver lipids peroxidation (Jose and Janardhanan, 2000; Bobek *et al.*, 2001 and Jayakumar *et al*., 2006).

Feeding mice with *P. ostreatus* decreases dramatically the food intake but increases the water intake and caused significantly increased in plasma alanine amino transferase activities. It seems that the doses and periods time required for the potential benefits of mushroom consumption to appear are similar to those causing undesirable effects. This should be taken into consideration if mushrooms are going to be used as functional food (Nieminen *et al.,* 2009).

Soy proteins, peptides and isoflavones may work together to produce effects on lipid metabolism and gene expressions. All potential mechanism of action has been suggested intestinal absorption of bile acids and dietary cholesterol, or on the hepatic metabolism of cholesterol and or lipoprotein (Anderson, 2003). Soy protein has lipid-lowering effect on hyperlipidemic hemodialysis patients. However, soy protein intake had little effect on plasma lipid levels in normolipidemic hemodialysis patient (Chen *et al*., 2005).

Beef burger prepared from meat with mushroom is considered a cheap high source of protein with low caloric value improving kidney function also; mushroom can be used as food additives to the childhood and aged diet (EL–Shewey 2001). Furthermore, mushroom can be used as a good fortification food ingredient (Abo EL-Naga, 2006). So, the aim of this research is studying the effect of feeding hypercholesterolemic rats on 35% beef patties fortified with 0, 4, 8 and 12% oyster dried mushroom on blood serum lipids profile as total cholesterol, triglycerides, total lipids, phospholipids, HDL, LDL, VLDL atherogenic index and liver enzymes as GOT and GPT comparing with feeding on 8% dried mushroom and 8% defatted soy flour diets.

MATERIALS AND METHODS

Materials

Imported Brazilian frozen beef meat, white rice, fresh mushroom, onions, and spices mixture were obtained from the local market of Mansoura city, Egypt. Defatted soy flour was obtained from Food Technology Research Institute, Agricultural Research Center, Giza, Egypt.

Male Albino rats (Sprague-Dawley) weighing 80 - 100 g, were obtained from the experimental animal house of Food Technology Research Institute, Agricultural Research Center, Giza, Egypt.

Kits of total lipids, total cholesterol, triglycerides, HDLc, GOT and GPT enzymatic colorimetric kits were obtained from Beta Company, Mansoura, Egypt.

Methods

Drying of mushroom:

Mushroom was washed by water, sliced, soaked in 1% brine solution containing 0.5% citric acid for 5 minutes and dried in electric oven at 50^{o} C for 12 hours, according to Mahdly (2001).

Preparation of beef patties

Beef patties used in this study consisted of 40% minced beef, 8% soy flour, 17% rice flour, 5% onion, 3.3% garlic, 1.4% salt, 1.8% spices mixture (25.5% coriander, 8% cubeb, 22.5 % cumin, 30% black pepper, 10% cardamom and 4% cinnamon) and 23.5% crush glace. Four beef patties were achieved by adding dried mushroom as following (0% control, 4, 8 and 12%). The more added mushroom the low rice was used. All beef patties were dried in electric oven at 70^{o} C till 5% moisture, according to EL-Shewey (2001), which was used in this dried form in feeding rats in the biological assay.

Chemical composition of beef patties defatted soy flour and dried mushroom:

Moisture, ash, crude protein and crude fat contents were determined according to the methods described by A.O.A.C (2005).Total carbohydrates were calculated by difference.

Biological assay

Animal and experimental design:

Fifty sex male Albino rats (Sprague -Dawley) weighing 80 - 100 g were kept under normal healthy conditions and fed on basal diet (experimental diets for adaptation) for seven days. Then, rats were divided randomly into eight groups (n = 7). Rats were fed on high fat diet for 15 days. Basal diet prepared according to Reeves, *et al.* (1993), additives to basal diet to prepare high fat diet prepared as described by Anderson, *et al*. (1994). All diets given in (Table 1). Dried beef patties were added to reach 12% level of protein. Rats were fed for 30 days according to the following scheme:-

G1- Rats fed on basal diet (negative control).
G2- Rats fed on high fat diet (positive control).
G3- Rats fed on high fat diet + 35 % beef patty with 0% dried mushroom.
G4-Rats fed on high fat diet + 35 % beef patties with 4% dried mushroom.
G5- Rats fed on high fat diet + 35 % beef patties with 8% dried mushroom.
G6- Rats fed on high fat diet + 35 % beef patties with 12% dried mushroom.
G7- Rats fed on high fat diet + 8% defatted soy flour.
G8- Rats fed on high fat diet + 8% dried mushroom.

During the experiment period (52 days), rats were kept separately in well aerated cages (stainless steel). The food intake and body weight were recorded every week and at the end of experiment. Feed efficiency ratio (FER) was calculated using the following equation as described by Ramadan (1995). FER = Gain body weight (g) / Feed intake (g).

Protein efficiency ratio PER was calculated using the following equation as described by Medany (2004) PER = Gain in body weight (g) / Protein intake (g).

Table (1): Composition of basal and high fat diets (g / 100 g).

Ingredients	Basal diet %	High fat diet %	High fat diets + beef patties %	High fat diets + Dried mushroom%	High fat diets + Defatted soy flour%
Casein	14	14	-	12	9.1
Dried beef patties	-	-	35	-	-
Dried mushroom	-	-	-	8	-
Defatted soy flour	-	-	-	-	8
Corn oil	4	-	-	-	-
Cellulose	5	5	5	5	5
Starch	72.30	55.05	34.05	49.05	51.95
Vitamins mixture	1	1	1	1	1
Salt mixture	3.5	3.5	3.5	3.5	3.5
Choline chloride	0.20	0.20	0.20	0.20	0.20
Animal fat	-	20	20	20.00	20.00
Cholesterol	-	1	1	1.00	1.00
Bile salt	-	0.25	0.25	0.25	0.25

After hypercholestrolimic period, after 15 days and at the end of experiment, rats were fasted overnight and anasthetized using diethyl ether and blood samples were taken from hepatic portal veins, the orbital venous plexuses by capillary tube into centrifuge tubes. Blood samples were centrifuged at 5000 r.p.m for 15 min to separate serum, and then kept in plastic vials at –20 ^{O}C until analysis. At the end of experiment heart, lung, liver, kidney, spleen and brain were removed and weighted to calculate its relative ratio to final body weight.

Relative organs weight was calculated as the following:

Relative organs weight = Organs weight (g) x 100 / Final body weight (g) described by Medany (2004).

Biochemical analysis of serum

- Serum total lipids (TL) were determined enzymatically according to the method described by Zollner and Kirsch (1962).
- The determination of total cholesterol (TC) in serum was performed according to the method described by Allain, *et al*. (1974).
- Triglycerides (TG) level was determined enzymatically according to the method described by Fossati and Prencipe (1982).
- The determination of HDL- cholesterol in plasma was performed according to the following colorimetric method of Naito and Kalpana (1984).
- The determination of LDL-c and vLDL-c in serum were performed according to the method of Lopez–Virella, *et al*. (1977).

Calculation of vLDL-c, LDL-c and phospholipids were carried out by the following equations: vLDL-c (mg/ dl) = TG /5.

LDL-c (mg/ dl) = TC– (vLDL-c + HDL-c).

Phospholipids = TL – (TG- TC).

- Atherogenic index was calculated according to Castelli and Levitar (1977).

Atherogenic index = Cholesterol / HDLc or LDLc / HDL

Glutamic oxaloacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT) were determined in serum samples using enzymatic colorimetric kits according to Reitman and Frankel (1957).

Statistical analysis

All data were statistically analyzed by applying SAS program (Statistical analysis system, 1999) using one-way analysis of variance (ANOVA). Dincans multiple range tests (1955) were used to compare between treatment means. Difference was considered significant at 0.05 probability level.

RESULTS AND DISCUSSION

Chemical composition of beef patties fortified with mushroom

Chemical composition of beef patties fortified with dried mushroom, defatted soy flour and dried mushroom were determined and presented in Table (2). The results indicate that moisture, carbohydrate contents of beef patties are decreased but protein, fat and ash contents are increased by increasing fortification with dried mushroom. This result may be due to dried mushroom had lower moisture and carbohydrates but it had higher protein, fat, and ash contents comparing to rice flour. In addition, defatted soy flour had the lowest content of moisture and the highest content of protein. Dried mushroom had the highest carbohydrates contents, but it had the lowest protein content comparing to all beef patties and defatted soy flour. All these results confirmed with those of Gallagher, *et al.* (2004) who reported that rice has low levels of sodium, protein, fat and fiber, and a high amount of easily digested carbohydrates. Also, Mattila *et al*., (2001) and Yang *et al*., (2001) they found that mushrooms provide a rich supply to the diet in the form of proteins, carbohydrates, valuable salts and vitamins. The protein value of mushroom is double of asparagus and potatoes, but lower than fish, snails' and broiler meat. Concerning on the chemical composition of dried mushroom is found to be in according to Hassan (2002). With respect to chemical composition of defatted soy flour the results confirmed with those of khalil, *et al.* (2002).

Table (2): Chemical composition of beef patties dried mushroom and defatted soy flour on dry weight basis.

Products	Moisture %	Protein %	Fat%	Ash%	Carbohydrate%
BPC	64.248 ± 0.06	36.835 ± 0.05	8.975 ± 0.01	6.738 ± 0.10	47.452 ± 0.154
BP1	63.867 ± 0.03	38.828 ± 0.06	9.340 ± 0.05	7.630 ± 0.21	44.202 ± 0.172
BP2	63.607 ± 0.08	40.169 ± 0.06	9.439 ± 0.09	8.472 ± 0.07	42.92 ± 0.139
BP3	63.487 ± 0.04	41.539 ± 0.03	9.574 ± 0.05	8.861 ± 0.06	40.026 ± 0.109
Dried mushroom	8.299 ± 0.022	21.854 ± 0.05	9.441 ± 0.103	8.486 ± .118	60.219 ± 0.198
Defatted soy flour	7.059 ± 0.054	52.25 ± 0.05	7.779 ± 0.02	8.263 ± .047	31.708 ± 0.031

BPC: Beef patty control fortified with 0% dried mushroom. BP1: Beef patty fortified with 4% dried mushroom. BP2: Beef patty fortified with 8% dried mushroom. BP3: Beef patty fortified with 12% dried mushroom. Each record is a mean value of three replicates and is followed by the stander deviation

Biological assay

Data Tabulated in Tables (3 and 4) show the effect of feeding on 35% beef patties forfeited with 0, 4, 8, and 12% dried mushroom, 8% defatted soy flour and 8% dried mushroom on feeding and growth parameters of hypercholestero-lemic rats such as daily food intake, final weight, body, daily body weight gain, food efficiency ratio and protein efficiency ratio. Data presented in Table (3) indicated that positive control group that fed on high fat diet has the lowest values of all parameters comparing to other groups. This result may be due to this diet caused an abnormal in metabolism of this group. On the other hand, group fed on 35% beef patty fortified with 0% dried mushroom has the highest values in daily food intake, body and daily weight gain comparing to groups fed on other beef patties. When the level of fortification with dried mushroom in beef patties preparation increased the levels of daily food intake, body and daily weight gain are decreased. Group fed on 8% defatted soy flour has the highest values in food efficiency ratio, protein efficiency ratio, body and daily weight gain comparing to other groups. This result may be due to protein of defatted soy flour is a complete protein. It was clear that there was no significant difference between group fed on 8% dried mushroom and group fed on basal diet in all parameters. Group fed on 8% dried mushroom has the lowest body weight gain comparing to other groups.

Table (3): Feeding and growth parameters of rats fed on beef patties fortified with dried mushroom

Groups	DFI	IW	FW	WG	DWG	FER	PER
Negative control	cb18.182 ± 1.46	a 916 ± 6.09	c 128 ± 9.03	c 36.4 ± 7.33	c 0.77 ± 0.16	c0.044 ± 0.01	d 0.36 ± 0.08
Positive control	c 15.63 ± 1.66	ba 84.4 ± 8.96	d 68.6 ± 12.01	d -15.8 ± 6.42	d -0.34 ± 0.14	d -0.02 ± 0.01	e -0.19 ± 0.09
35%BPC	a 25.31 ± 2.56	ba 85.6 ± 8.35	a 177.4 ± 26.34	a 91.8 ±22.93	a 1.96 ± 0.49	b 0.08 ± 0.02	ba 0.65 ± 0.17
35%BP1	a 24.13 ±3.16	a 89.4 ± 7.44	a 177.4 ± 27.65	a 88 ± 23.08	a 1.87 ± 0.49	b 0.08 ± 0.02	ba 0.65 ± 0.14
35%BP2	a 24.23 ± 3.06	a 90.4 ± 5.86	a 178.2 ± 35.37	a 87.8 ± 35.76	a 1.87 ± 0.76	b 0.08 ± 0.03	bac 0.59 ± 0.24
35%BP3	a 23.55 ± 0.45	ba 88 ± 6.63	ba165.75 ± 4.49	ba7.75 ± 9.96	ba1.65 ± 0.21	b 0.07 ± 0.01	bdc 0.52 ± 0.07
8%Defatted soy flour	b 19.51 ± 0.66	c 74.4 ± 3.97	ba 66.8 ± 17.24	a 92.4 ± 18.81	a 1.97 ± 0.4	a 0.1 ± 0.02	a 0.72 ± 0.15
8% Dried mushroom	b 19.58 ± 0.77	bc 79.8 ± 7.09	bc 40.2 ± 14.17	bc 60.4 ± 17.4	bc1.29 ± 0.37	cb .06 ± 0.01	dc 0.45 ± 0.12
P<	0.0001	0.0072	0.0001	0.0001	0.0001	0.0001	0.0001
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BPC: Beef patty control fortified with 0% dried mushroom. BP1: Beef patty fortified with 4% dried mushroom. BP2: Beef patty fortified with 8% dried mushroom. BP3: Beef patty fortified with 12% dried mushroom. DFI: Daily Food intake. IW: Initial weight (g). FW: Final weight. WG: weight gain. DWG: Daily weight gain. FER: Food efficiency ratio. PER: Protein efficiency ratio. Each value is the mean ± SD, n: 5/ group. (a, b, c, d, e, and f): means in the same column with different superscript differ significantly at p< 0.05. *P< 0.05. **P< 0.01. *** P< 0.001. ****P< 0.0001

All these results are in harmony with those of EL-Bastawesy and Hareedy (2004), who observed that rats fed on high fat diet had a significant decrease in body weight gain and decrease in food intake. In addition, with respect to soy protein, these results are in conformity with those of Hurley, *et al.* (1998) who found that feeding on soy protein isolate-cornstarch caused reduced food intake and body weight gain. By concerning to feeding on dried mushroom, these results are in accordance to those of Jeong, *et al*. (2010) who indicated that body weight, food intake, and food efficiency ratios were similar to mushroom Agaricus *bisporus* powder fed and control hypercholesterolemic rats except for lower food intakes, body weights and food efficiency ratios in hypercholesterolemic rats were significantly higher than those in normal rats.

Data in presented Table (4) show the effect of different diets on organs weight, such as heart, lung, liver, kidney, spleen and brain and also the ratio between organs weight to its body weight of experimental hypercholesterolemic rats. The results show that there were no differences between positive control and negative control groups in all weight organs, but there were differences in relative weight of all organs between both groups except spleen organs.

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The highest relative weights of all organs were found in positive control group comparing to others. These result may be due to positive control group had a decrease in final body weight comparing to other groups as a result to hypercholesterolemic case which caused an abnormality in metabolism in body and organs as recorded in Table (3). Moreover, the result indicated that the lowest weight relative organs as lung was found in group fed on beef patty forfeited with 8% dried mushroom. Furthermore, the lowest relative weight of kidney was observed in rats fed on beef patty forfeited with 12% dried mushroom.

Furthermore, spleen, brain and heart were found in rats fed on beef patty fortified with 4% dried mushroom comparing to other groups. Moreover, all hypercholesterolemic groups had fatty livers comparing to negative control group. On the other hand group fed on 8% dried mushroom had the lowest ratio of liver followed by group fed on beef patty fortified with 12% dried mushroom comparing to other hypercholesterolemic groups. All these results are in concurrence with those of Park, *et al*. (1998) and Jeong, *et al*. (2010) stated that liver weight and liver to body weight ratio were significantly greater in rats fed on high fat diet than those fed basal diet. In addition, Ramadan (1995) found that dried mushroom caused decreases in liver weight and liver ratio. Additionally, Morsi (2003) reported that defatted soy flour had hypolipimic and hypocholestorlimic effects. Liver of bioactive protein had the lowest liver weight compared to hypercholesterolemic group.

Data presented in Tables (5 and 6) show that serum blood lipids profile of rats after hypercholesterolemic period and after feeding on 35% dried beef patties fortified with 0, 4, 8 and 12% dried mushroom, 8% defatted soy flour and 8% dried mushroom. From these results, it could be seen that, after hypercholesterolemic period, all groups feeding on high fat diet had a significant increase in serum blood lipids comparing to negative control group.

After 15 days, there was significant increase in total cholesterol, triglycerides and total lipids levels of positive control group, groups fed on 35% beef patty forfeited with 0 and 4% dried mushroom, and also total cholesterol is increased in group fed on 35% beef patty forfeited with 8% dried mushroom comparing to after hypercholesterolemic period and compared to other groups, which they had decreases. On the other hand all groups had a decrease in phospholipids level except group fed on 8% defatted soy flour compared to after hypercholesterolemic period and other groups after 15 days. This result is due to this group had high total lipids level compared to others. So, these time and dose of feeding are enough to cause high hypercholesterolemic lowering effect in phospholipids Furthermore, the result cleared that all hypercholesterolemic groups had significant reduction in all levels of serum lipids comparing to positive control group after 30 days. This result may be due to increase the hypercholesterolemic lowering effect of these diets as result to increase in feeding time. The highest hypercholesterolemic lowering effect on total cholesterol, total lipids and phospholipids were found in group fed on 35% beef patty forfeited with 12% dried mushroom. In addition, the highest hypercholesterolemic lowering effect in triglycerides was found in group fed on 35% beef patty forfeited with 8% dried mushroom followed by group fed on 35% beef patty forfeited with 12% dried mushroom. Also, the reduction in total cholesterol and triglycerides on group fed on 35% beef patty forfeited with 0% dried mushroom is may be due to hypercholesterolemic lowering effect of defatted soy flour plus other additives as garlic and onion. Furthermore, there were no significant reduction in all serum lipids between groups fed on 8% defatted soy flour and 8% dried mushroom after 30 days. Moreover, hypercholesterolemic lowering effect of groups fed on 8% dried mushroom equal the effect of group fed on 8% defatted soy flour and 35% beef patty fortified with 0% dried mushroom but not more than the effect lowering of groups fed on other 35% beef patties. This result may be due to there are fiber and other compounds as photochemical in defatted soy flour and dried mushroom which had hypercholesterolemic lowering effect in serum lipids.

Table (5): Serum total cholesterol TC and triglycerides TG (mg/dl) of control and experimental hypercholesterolemic rats fed on beef patties fortified with dried mushroom.

Groups	Total Cholesterol (TC)				Triglycerides (TG)			
	After HCH period	Repletion During period		Redu-ction	After HCH period	Repletion During period		Reduct-ion
		15 days	30 days			15 days	30 days	
Negative control	e 95.73 ± 8.25	e 90.61 ± 0.36	c 87.21 ± 2.05	d9.21 ± 4.79	e66.6 ± 8.32	c 65.40 ± 9.21	cb 63.6 ± 2.06	e 5.06 ± 6.02
Positive control	c 151.73 ± 9.35	a 228.3 ± 6.27	a 251.9 ± 5.26	e -66.64 ± 2.85	cd137.5 ± 0.79	a144.8 ± 8.23	a162.72 ± 9.37	f -18.51 ± 3.89
35% BPC	c 153.53 ± 5.6	a 233.44 ± 7.06	b 125.42 ± 6.85	cd 18.34 ± 2.24	cb 142 ± 7.87	a 144.16 ± 8.15	b 72.42 ± 9.91	d 49.07 ± 5.59
35% BP 1	a 176.13 ± 8.13	a 226.76 ± 0.32	b 120 ± 7.97	cb 31.81 ± 4.44	cd 135 ± 8.37	a 138.94 ± 0.06	b 67.80 ± 9.07	d 49.84 ± 5.12
35% BP2	d 135.33 ± 3	b 167.03 ± 9.53	c 90.66 ± 6.39	cb 32.97 ± 5.07	a 163.2 ± 7.5	b 112.34 ± 9.44	d 50.20 ± 9.18	a 69.37 ± 4.26
35% BP3	b 165.4 ± 9.46	d 116.55 ± 5.77	d 73.64 ± 7.77	a 55.56 ± 2.53	d 127 ± 8.78	c 73.85 ± 9.77	d 46.50 ± 8.02	bc 63.318 ± 6.59
8% Defatted soy flour	d 140.93 ± 4.91	c 129.41 ± 8.42	c86.5 ± 5.85	b 38.59 ± 4.17	d 128.2 ± 9.63	b120.78 ± 8.61	cd 54.40 ± 4.56	bc 57.55 ± 2.02
8% Dried mushroom	a 178.27 ± 6.07	dc 127.48 ± 5.49	c 91.04 ± 8.89	b 35.94 ± 3.98	b 151.4 ± 8.26	a 134.95 ± 8.90	B 68.80 ± 7.40	dc 54.42 ± 5.84
P<	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
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BPC: Beef patty control fortified with 0% dried mushroom. BP1: Beef patty fortified with 4% dried mushroom. BP2: Beef patty fortified with 8% dried mushroom. BP3: Beef patty fortified with 12% dried mushroom. HCH: hypercholesterolemic. Each value is the mean ± SD, n: 5/ group. (a, b, c, d, e, and f): means in the same column with different superscript differ significantly at p< 0.05. *P< 0.05. **P< 0.01. *** P< 0.001. ****P< 0.0 001

All these results consent with those of EL-Bastawesy and Hareedy (2004) and Kumar, *et al*. (2009) who indicated that hepatic and serum total cholesterol, triglycerides, phospholipids and total lipids levels of rats fed on a high fat diet containing cholesterol and/or bile salts were significantly increased in hypercholesterolemic comparing to control animals receiving vehicle or normal diet. With revere to mushroom the obtained results are in agreement with results observed by Medany (2004) who reported that feeding hypercho-lesterolemic rats with 3 or 5% dried mushroom effectively lower total cholesterol serum after one week. By continuing feeding the reduction was continuing in hypercholesterolemic rats comparing to rats fed on basal diet. Furthermore, the present results are in agreement with those of Mishra and Singh (2010), who found that total lipids content significantly (P< 0.05) was reduced about 7.06% in albino rats fed on diets supplemented with 15% mushroom. In addition, these results are in harmony with those of Effraim, *et al*. (2000) who indicated that garlic (raw or extracted oil) possess possible cholesterol, triglycerides, blood glucose level, lowering activity. Furthermore, with revere to the result of defatted soy flour, it agrees with that of Potter (1998) who found that isoflavones may act as anti-oestrogenc, iso-flavones alone will not decrease blood lipid levels as described but influence the ability of soy protein products to reduce serum lipid concentrations.

Table (6): Serum total lipids and phospholipids of control and experimental hypercholesterolemic rats fed on beef patties fortified with dried mushroom.

Groups	Total Lipids				Phospholipids			
	After HCH period	Repletion During period		Reduc-tion	After HCH period	Repletion During period		Reduc-tion
		15days	30 days			15 days	30 days	
Negative control	c 512.3 ± 36.65	c 484.24 ± 7.37	cd451.94 ± 0.21	e11.27 ± 9.26	c 349.95 ± 8.88	c 328.2 ±19.63	ed 01.12 ± 23.07	b12.78 ± 1.78
Positive control	b 677.24 ± 54.2	a 750.82 ± 2.44	a 901.76 ± 5.42	f -33.65 ± 1.45	cb 387.96 ± 5.98	bc 377.71 ±101.3	a 487.13 ± 69.55	c -27.73 ± 21.7
35% BPC	b 652.12 ± 54.23	a 716.16 ±70.24	b 531.22 ± 8.84	ed 18.55 ± 2.81	c 356.57 ± 9.03	c 338.54 ±66.51	cbd333.4 ± 50.29	b6.05 ± 6.91
35% BP 1	b 665.98 ± 39.90	a 713.62 ± 0.34	cb 499.98 ±18.72	cd 24.84 ± 1.97	c 354.83 ± 0.36	c 347.9 ±30.47	ced 12.18 ± 16.87	b11.55 ± 5.48
35% BP2	b 639.02 ± 58.62	b 617.9 ± 64.3	d 403.88 ±46.84	B 36.834 ± 3.55	c 340.49 ± 7.06	c338.53 ±70.07	e 263 ± 54.89	b22.88 ± 7.71
35% BP3	a 780.9 ± 68.36	b 592.28 ± 6.28	e 265.6 ± 2.59	a66.19 ± 4.17	a 488.5 ±69.40	bac401.89 ±30.27	f 145.46 ± 61.35	a 70.96 ± 8.68
8%Defatted soy flour	b 688.58 ± 59.23	a 713.34 ± 4.83	b 528.92 ±53.39	cd22.34 ± 3.23	bac 19.42 ± 1.29	a463.12 ±72.69	b388.04 ± 50.64	b5.00 ± 3.28
8% Dried mushroom	a 780.08 ± 89.36	a 699.68 ± 62.1	b 531.58 ± 9.87	cb31.07 ± 9.24	ba 450.4 ± 3.99	ba 438.22 ±64.43	cb 371.74 ± 37.89	b14.82 ± 9.20
P<	0.0001	0.0001	0.0001	0.0001	0.004	0.0097	0.0001	0.0001
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BPC: Beef patty control fortified with 0% dried mushroom. BP1: Beef patty fortified with 4% dried mushroom. BP2: Beef patty fortified with 8% dried mushroom. BP3: Beef patty fortified with 12% dried mushroom. HCH: hypercholesterolemic. Each value is the mean ± SD, n: 5/ group. (a, b, c, d, e, and f): means in the same column with different superscript differ significantly at p< 0.05. *P< 0.05. **P< 0.01. *** P< 0.001. ****P< 0.0 001

From data listed in Table (7) it could be noticed that after hyperch-olesterolemic period, there is significant decrease in HDL-c levels, but there were significant increases in LDL-c and vLDL-c levels of all hypercholesterolemic groups comparing to negative control group.

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After 15 days, there were significant increases in HDL-c, and decreases in LDL-c levels in all hypercholesterolemic rats except groups fed on high fat diet, beef patties forfeited with 0 or 8% dried mushroom had continuous slightly an increase in vLDL-c levels comparing to other groups and after hypercholesterolemic period, this result may be due to the hypercholesterolemic lowering effect of these diets was not enough to cause reduction after this time of feeding. In addition, after 30 days, there was a significant increase in HDL-c level; however there were significant reduction in LDL-c and VLDL-c levels in all hypercholester-olemic groups comparing to positive control group which had continuous decrease in HDL-c and increases in LDL-c and VLDL-c levels. Also, groups fed on 35% beef patties forfeited with 0 and 4% dried mushroom showed the highest increase in HDL-c comparing to other groups. This result due to these groups had the highest levels in total cholesterol as shown in Table (5).

Group fed on 35% beef patties forfeited with 12% dried mushroom showed the highest reducing in LDL-c level followed by group fed on 8% dried mushroom and group fed on 8% defatted soy flour. Group fed on 35% beef patties forfeited with 8% dried mushroom showed the highest reducing in vLDL-c level comparing to others after 30 days. This result is due to that this group had reduction on triglycerides at this period as in Table (5). Moreover, these results showed that groups fed on defatted soy flour and dried mushroom had the same effect of increasing in HDL-c and reduction in HDL-c and VLDL-c levels in addition, both groups had the lowest levels in LDL-c comparing to group fed on 35% beef patty forfeited and 8% dried mushroom. This result may be due to the effect of reducing which was used to avoid the risk of meat.

All these results are harmony with that of Woo, *et al*. (2009) who reported that hyperlipidemia caused an increase in serum VLDL, LDL and caused a decrease in HDL. Furthermore, with respect to the results of mushroom they have conformity with those of Mattila (2002), who stated that the most abundant phytosterol in mushrooms is ergosterol. The nutritional interest in phytosterols derives from the fact that reduces the absorption of cholesterol, there by having the capacity to lower plasma cholesterol and LDL–c with no detrimental side-effects. In addition, the present results of defatted soy flour appeared to agree with those of Morsi (2003), Cupisti, *et al*. (2004), Tovar, *et al.* (2002) and Torres, *et al*. (2006) who reported that soy protein compared to casein decreases cholesterol absorption but increases cholesterol degradation to form bile acid. In addition, soy protein increases (LDL) receptor activity

Lipids and lipoproteins are well known risk factors for ischemic heart disease. Elevated levels of triglyceride, cholesterol and LDL-C are documented as risk factors for atherogenesis (Anon, 1984). Data in Table (8) cleared that the effect of feeding on different diets on atherogenic index as CH/HDL-c and LDL/HDL-c of hypercholesterolemic rats after 15 and 30 days. After hyperchol-esterolemic period, there was significant increase on atherogenic index of all hypercholesterolemic groups comparing to negative control group. This result is due to an increase in total cholesterol and LDL-c and decrease in HDL-c in these groups as aforementioned. So, all hypercholesterolemic groups had high hypercholesterolemic risk of heart diseases comparing to negative control group. After 15 and 30 days, all hypercholesterolemic groups had significant reduction in atherogenic index except positive control group which had continuous an increase. The lowest values of atherogenic index were found in group fed on 35%beef patties fortified 12% dried mushroom followed by group fed on 8% dried mushroom and 8% defatted soy flour, respectively, as well, both groups fed on 8% dried mushroom and 8% defatted soy flour groups had the same hypercholesterolemic lowering effect. All these results are confirmed with those obtained by Aronow, (2006) reported that hyperlipidemia is commonly associated with atherosclerotic vascular diseases and is present as a risk factor for coronary heart disease. In addition, Damasceno, *et al*. (2001) indicated that soy protein isolate, in comparison with casein, promoted a decrease of atherogenic lipoproteins (β-VLDL and LDL), which had beneficial effects over atherosclerosis progression in cholesterol-fed rabbits. Besides, Alam, *et al.* (2007) reported that feeding hypercholesterol-aemic rats on 5% oyster mushroom supplementation provides health benefits, at least partially, by acting on the atherogenic lipid profile in the hypercholesterolemic condition.

Table (8): Cholesterol /HDL-c (mg/dl) and LDL/HDL-c (mg/dl) of control and hypercholesterolemic rats fed on beef patties fortified with dried mushroom.

Groups	CH/HDL-c (mg/dl)				LDL/ HDL-c (mg/dl)			
	After HCH Period	repletion During period		Reducing	After HCH period	repletion During period		Reducing
		15 days	30 days			15 days	30 days	
Negative control	c 1.41 ± 0.09	b1.39 ± 0.14	b1.39 ± 0.17	b0.64 ±14.23	d0.21 ± 0.07	b0.18 ± 0.14	b0.191 ± 0.15	a -4.16 ± 87.92
Positive control	ba 3.21 ± 0.68	a6.54 ± 1.37	a10.82 ± 2.31	c -258.42 ±140.24	bac 1.63 ± 0.59	a 4.72 ± 1.25	a8.43 ± 2.07	b -502.84 ± 26.28
35% BPC	b 2.99 ± 0.36	b1.29 ± 0.04	b1.40 ± 0.09	ba 2.64 ± 4.55	bc1.43 ± 0.28	b0.13 ± 0.04	b 0.24 ± 0.06	a83.24 ± 3.28
35% BP 1	a 3.67 ± 0.81	b1.26 ± 0.02	b 1.30 ± 0.11	ba3.8 ± 4.9	a 2.11 ± 0.7	b0.11 ± 0.01	b 0.16 ± 0.11	a 93.15 ± 2.75
35% BP2	b 2.8 ± 0.35	b 1.31 ± 0.06	b 1.30 ± 0.05	ba 52.88 ± 4.77	c1.12 ± 0.27	b0.14 ± 0.04	b 0.16 ± 0.03	a85.62 ± 1.7
35% BP3	ba3.44 ± 0.59	b 1.18 ± 0.02	b1.17 ± 0.04	a65.23 ± 6.04	ba 1.91 ± 0.49	b 0.03 ± 0.01	b0.02 ± 0.004	a98.92 ± 0.37
8% Defatted soy flour	ba3.15 ± 0.61	b 1.31 ± 0.05	b 1.27 ± 0.05	ba58.61 ± 7.66	bac1.58 ± 0.51	b0.06 ± 0.02	b0.11 ± 0.03	a92.79 ± 2.41
8% Dried mushroom	ba 3.3 ± 0.18	b 1.36 ± 0.07	b 1.25 ± 0.21	ba 61.92 ± 5.98	ba1.74 ± 0.16	b0.07 ± 0.04	b0.06 ± 0.18	a 96.52 ± 9.82
P<	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
	***	***	***	***	***	***	***	***

BPC: Beef patty control fortified with 0% dried mushroom. BP1: Beef patty fortified with 4% dried mushroom. BP2: Beef patty fortified with 8% dried mushroom. BP3: Beef patty fortified with 12% dried mushroom. HCH: hypercholesterolemic. Each value is the mean ± SD, n: 5/ group. (a, b, c, d, e, and f): means in the same column with different superscript differ significantly at p< 0.05. *P< 0.05. **P< 0.01. *** P< 0.001. ****P< 0.0 001.

Data tabulated in Table (9) showed that the effect of beef patties fortified with different levels of dried mushroom, 8% dried mushroom and 8% defatted soy flour on hepatic markers as GOT and GPT of hypercholesterolimic rats after feeding for 15 and 30 days. After hypercholesterolemic period, there was significant increase in GPT and GOT enzymes of all hypercholesterolemic groups comparing to negative control group. This result may be due to all hypercholes-terolemic groups had fatty livers which led to increase in serum liver enzymes, which are usually indicative of possible liver damage. After 15 and 30 days, all hypercholesterolemic rats had significant reduction in GOT and GPT enzymes except positive control group which had continuous increase. The highest values of GOT and GPT enzymes were found in group fed on 35% beef patty fortified with 0% dried mushroom compared to other groups. The lowest values of GOT and GPT enzymes were found in group fed on 35% beef patty fortified with 12% dried mushroom at two period followed by group fed on 35% beef patty fortified with 8% dried mushroom. Groups fed on 8% dried mushroom and 8% defatted soy flour had the same hypercholesterolemic lowering effect equal the effect of beef patty fortified with 4% dried mushroom.

Table (9): Serum GPT (IUI/I) and GOT (IUI/I) of control and hypercholesterolemic rats fed on beef patties fortified with dried mushroom

Groups	GPT (IUI/I)				GOT (IUI/I)			
	After HCH period	repletion During period		Reduction	After HCH period	Repletion During period		Reduction
		15 days	30 days			15 days	30 days	
Negative control	b 24.4 ± 8.65	d20 ± 4.64	cb 21.2 ± 5.26	d8.12 ± 24.8	b22.2 ± 2.49	cb 22 ± 4.06	b20 ± 3.39	d10.17 ± 8.01
Positive control	a 46.8 ± 1.3	a62.2 ± 9.96	a 82 ± 4.3	e-75.18 ± 6.95	a 31.8 ± 4.76	a40.2 ± 2.17	a 46.8 ± 1.64	e -50.21 ± 25.74
35% BPC	a 44.4 ± 1.67	b 34.8 ± 4.49	b24.4 ± 4.16	c45.1 ± 8.77	a 31.2 ± 3.11	b 25 ± 2.24	b 21.2 ± 4.21	c32.08 ± 10.89
35% BP1	a 47.6 ± 1.14	Cb 29.2 ± 4.44	cb20.8 ± 5.07	bac56.24 ± 0.78	a 31.4 ± 2.07	cd9.2 ± 1.92	c 15.2 ± 1.92	b51.67 ± 4.61
35% BP2	a45.8 ± 2.17	Cd 25.4 ± 2.19	cd17.8 ± 1.92	Ba 60.93 ± 5.79	a 31.4 ± 1.52	ed 16.4 ± 1.14	c12.4 ± 1.14	ba0.48 ± 3.52
35% BP3	a46 ± 2.83	d 21.6 ± 1.14	d 14.6 ± 2.02	a68.33 ± 3.38	a31.6 ± 5.03	e13.6 ± 1.52	d8.6 ± 0.89	a 72.23 ± 4.98
8% Defatted soy flour	a47.1 ± 2.19	b32.8 ± 5.54	cb20.6 ± 3.65	Bac56.03 ± 9.26	a29.6 ± 1.82	c20.4 ± 2.88	c 14.8 ± 2.28	b 49.67 ± 9.49
8% Dried mushroom	a 45.8 ± 4.55	b 34 ± 3.67	cb 22.6 ± 1.14	bc50.08 ± 7.56	a 29.2 ± 2.59	e 16 ± 2.35	c 13.2 ± 1.79	b 54.26 ± 9
P<	0.0001	0.0001	0.0001	0.0001	0.0005	0.0001	0.0001	0.0001
	****	****	****	****	***	****	****	****

BPC: Beef patty control fortified with 0% dried mushroom. BP1: Beef patty fortified with 4% dried mushroom. BP2: Beef patty fortified with 8% dried mushroom. BP3: Beef patty fortified with 12% dried mushroom.. HCH: hypercholesterolemic. Each value is the mean ± SD, n: 5/ group. (a, b, c, d, e, and f): means in the same column with different superscript differ significantly at p< 0.05. *P< 0.05. **P< 0.01.

All these results obtained are to be according to Hossain, *et al.* (2003), Giboney (2005), Woo, *et al*. (2009) and Oboh and Olumese (2010) who reported that hyperlipidemic may be responsible for liver damage in animal models of high-fat diet. With rever to the results of soy protein these results are in agreement with those of Aoyama, *et al.* (2000) who found that soybean protein has a hypercholesterolemic lowering effect compared to animal proteins such as casein. Also, it markedly decreases the activity of hepatic lipogenic enzymes. With respect to the results of dried mushroom they are in agreement with those of Choudhury, *et al.* (2009) who found that the feeding of fresh oyster mushroom at the Ifter table during Ramadan fasting significantly decreased the serum levels of GPT and GOT as compared to those of the control subjects. These results suggest that *P. ostreatus* may be able to ameliorate hepatocellular functions significantly.

Conclusion

All beef patties fortified with dried mushroom, dried mushroom and defatted soy flour had high hypercholesterolemic lowering effect on total cholesterol, triglycerides, total lipids, phospholipids, LDL-c and VLDL-c, , atherogenic index and liver enzymes GOT and GPT of hypercholesterolemic rats after 30 compared to 15 days. Moreover, by increasing levels of fortification beef patties with dried mushroom the lowering hypercholesterolemic effect beef patties were increased.

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تأثير التغذية على أقراص اللحم المدعمة بعيش الغراب على صورة ليبيدات الدم وإنزيمات الكبد للفئران المصابة بارتفاع الكوليسترول في الدم

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تم إجراء هذا البحث لدراسة مدى تأثير التغذية بوجبات محتوية على ٣٥% أقراص لحم مدعمة بعيش غراب محاري مجفف بنسب صفر, ٤, ٨ و ١٢% على صورة ليبيدات الدم وإنزيمات الكبد للفئران المصابة بارتفاع الكوليسترول في الدم, ومقارنتها بالتغذية على وجبات محتوية على ٨% عيش غراب جاف و ٨% دقيق صويا منزوع الدهن. أوضحت النتائج أن كل الوجبات أظهرت أعلى تأثير خافض الكوليسترول المرتفع في الدم بعد التغذية لمدة ٣٠ يوم بالمقارنة بعد ١٥ يوم. كما أظهرت المجموعة المغذاة على ٣٥% قرص اللحم المدعم بعيش غراب بنسبة ١٢% اعلي معدل انخفاض في الكوليسترول الكلى, الجليسريدات الثلاثية, الليبوبروتينات منخفضة الكثافة, الليبوبروتينات منخفضة جدا في الكثافة, الليبيدات الكلية الفسفوليبيدات, بينما كان لديها أعلى معدل ارتفاع في الليبوبروتينات مرتفعة الكثافة عند مقارنتها بباقي المجموعات. ولذلك فان هذه المجموعة لديها اقل مؤشرات للإصابة بتصلب الشرايين مثل مؤشر (الكوليسترول/ الليبوبروتينات مرتفعة الكثافة) ومؤشر (الليبوبروتينات منخفضة الكثافة/ الليبوبروتينات مرتفعة الكثافة) بالمقارنة بباقي المجموعات الأخرى. ومن جانب إنزيمات الكبد GOT و GPT كانت المجموعة المغذاة على ٣٥% قرص لحم المدعمة بعيش غراب بنسبة ١٢% لديها أعلى معدل انخفاض. هذه النتائج ترجع إلى أن عيش الغراب المكون لأقراص اللحم لدية قدرة عالية على خفض الكوليسترول المرتفع في الدم. كانت المجموعات المغذاة على ٨% عيش غراب جاف و ٨% دقيق صويا منزوع الدهن لديها نفس التأثير الخافض على صورة ليبيدات الدم و إنزيمات الكبد. كما أن التأثير الخافض الكوليسترول المرتفع يزداد بزيادة التدعيم لأقراص اللحم بعيش الغراب الجاف, كما يرتفع أيضا بزيادة فترة التغذية.

قام بتحكيم البحث

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Table (4): Organs weight and relative weight of control and experimental hypercholesterolemic rats fed on beef patties fortified with dried mushroom.

Groups	Body weight	Heart	Relative	Lung	Relative	Liver	Relative	Kidney	Relative	Spleen	Relative	Brain	Relative
Negative control	c 128 ± 9.03	b 0.4 ± 0.07	b 0.312 ± 0.04	dc 1.02 ± 0.31	b 0.79 ± 0.22	c 4.3 ± 1	d 3.32 ± 0.58	c 1.1 ± 0.2	c 0.82 ± 0.13	ba 0.72 ± 0.38	ba 0.55 ± 0.93	d 1.28 ± 0.08	b .01 ± 0.10
Positive control	d 68.6 ±12.01	b 0.46 ± 0.08	a 0.68 ± 0.16	d 0.89 ± 0.04	a,1.32 ± 0.18	c 5 ± 0.4	a 7.52 ± 1.62	bc 1.3 ± 0.1	a 1.93 ± 0.3	b 0.48 ± 0.66	a 0.70 ± 0.23	bcd1.39 ± 0.1	a 2.05 ± 0.23
35% BPC	a177.4 ± 6.34	a 0.65 ± 0.07	b 0.37 ± 0.08	a 1.37 ± 0.28	b 0.79 ± 0.23	a 10 ± 1.5	b 5.8 ± 1.78	ba 1.5 ± 0.2	cb 0.89 ± 0.26	b 0.52 ± 0.1	ba 0.29 ± 0.05	bcd 1.37 ± 0.13	c 0.78 ±0.14
35% BP 1	a 77.4 ±27.65	a 0.6 ± 0.09	b 0.34 ± 0.02	a 1.34 ± 0.3	b 0.76 ± 0.2	a 9.1 ± 1.1	cb 5.18 ± 0.74	ba 1.6 ±0.3	cb 0.89 ± 0.16	b 0.4 ± 0.06	b 0.22 ± 0.02	cd 1.31 ±0.11	c 0.74 ± 0.11
35% BP2	a 78.2 ± 5.37	a 0.63 ± 0.07	b 0.36 ± 0.06	Bac 1.196 ±0.21	b 0.7 ± 0.21	a 9 ± 2.2	cb 5.28 ± 1.83	a 1.8 ± 0.7	b 1.06 ± 0.42	b 0.42 ±0.06	ba 0.24 ± 0.06	a 1.58 ± 0.12	cb 0.91 ± 0.23
35% BP3	ba165.75 ± 4.49	a 0.61 ± 0.09	b 0.37 ± 0.06	bac 1.25 ±0.2	b 0.75 ±0.11	b 6.9 ± 0.9	cd 4.17 ± 0.48	bc 1.2 ± 0.1	c 0.75 ± 0.04	ba 0.64 ±0.1	ba 0.38 ± 0.06	bc 1.43 ±0.11	cb 0.86 ±0.04
8%Defatted soy flour	ba 66.8 ± 7.24	a 0.64 ± 0.06	b 0.39 ± 0.03	ba1.318 ± 0.07	b 0.79 ± 0.06	a 9.5 ± 0.7	b 5.69 ± 0.36	bc 1.3 ± 0.1	cb 0.78 ± 0.05	b 0.41 ± 0.03	ba 0.24 ± 0.01	ba 1.5 ± 0.04	cb 0.90 ± 0.09
8% Dried mushroom	bc 0.2 ± 4.17	a 0.55 ± 0.05	b 0.4 ± 0.08	bdc 1.058 ± 0.105	b 0.75 ± 0.02	cb 5.7 ± 0.5	cd 4.04 ± 0.06	bc 1.2 ± 0.1	cb 0.86 ± 0.03	a 0.97 ± 0.39	ba 0.67 ± 0.22	bcd 1.38 ± 0.11	b 0.99 ± 0.10
P<	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
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BPC: Beef patty control fortified with 0% dried mushroom. BP1: Beef patty fortified with 4% dried mushroom. BP2: Beef patty fortified with 8% dried mushroom. BP3: Beef patty fortified with 12% dried mushroom.. Each value is the mean ± SD, n: 5/ group. (a, b, c, d, e, and f): means in the same column with different superscript differ significantly at p< 0.05. * P< 0.05. **P< 0.01. *** P< 0.001. ****P< 0.0 001

Table (7): Serum HDL-c and LDL and VLDL-c (mg/dl) of control and experimental hypercholesterolemic rats fed on beef patties fortified with dried mushroom.

Groups	HDL-c (ml/ dl)				LDL-c(ml/ dl)				VLDL-c (ml/ dl)			
	Period	During repletion period		Increa s-ing	HCH period	During repletion period		Reduct ion	HCH period	During repletion period		Redu-ction
		15 days	30 days			15 days	30 days			15 days	30 days	
Negative control	a68.03 ± 5.98	d 65.47 ± 6.51	c62.64 ± 5.96	b7.38 ± 11.75	d14.38 ± 4.35	cd 12.06 ± 8.98	cbd11.85 ± 9.85	b-11.51 ± 67.86	e 13.32 ± 1.66	c 13.08 ± 1.84	cb12.72 ± 2.41	E5.06 ± 6.02
Positive control	b48.7 ±8.62	e 36 ± 6.78	d23.95 ± 3.94	a48.83 ± 15.4	b75.53 ± 15.62	a163.43 ± 18.94	a 195.41 ± 9.91	c69.15 ± 64.43	cd 7.50 ± 2.16	a 28.96 ± 1.65	a2.54 ± 1.87	F8.51 ± 3.89
35% BPC	b 52.03 ± 6.65	a 180.60 ± 5.03	a89.71 ± 7.67	de-73.91 ± 19.97	b73.1 ± 6.8	b24.01 ± 6.68	b 21.22 ± 4.73	a-71.18 ± 4.6	cb 28.40 ± 1.57	a 28.83 ± 1.63	b4.48 ± 1.98	D49.06 ± 5.59
35% BP 1	b 49.47 ±9.22	a 180 ± 9.35	a92.4 ± 4.42	e-92.24 ± 37.91	a99.66 ± 12.89	cb18.97 ± 1.13	cb14 ± 9.66	a-6.35 ± 7.7	cd27 ± 1.67	a 27.79 ± 2.01	b3.56 ± 1.81	D49.84 ± 5.12
35% BP2	b48.98 ± 5.77	b 127 ± 10.3	cb69.62 ± 6.18	dc-43.27 ± 15.77	c53.71 ± 7.01	cb17.17 ± 4.33	ced11 ± 1.84	a-79.54 ± 2.08	a2.64 ± 1.5	b 22.46 ± 1.89	d10.04 ± 1.84	A69.37 ± 4.26
35% BP3	b49.22 ± 9.31	c 99.2 ± 5.36	c 63.12 ± 8.12	c-30.43 ± 20.49	a90.78 ± 11.79	d2.58 ± 1.16	e1.22 ± 0.23	a-984.6 ± 0.28	d25.4 ± 1.76	c 14.77 ± 1.95	d9.30 ± 1.60	Ba63.32 ± 6.59
8% Defatted soy flour	b46.02 ± 8.37	c 99 ± 9.19	cb68.42 ± 6.96	dc-53.87 ± 38.27	b69.28 ± 8.68	d6.25 ± 1.85	ced 7.2 ± 1.53	a89.53 ± 2.08	d5.64 ± 1.93	b 24.16 ± 1.72	cd10.88 ± 0.91	Bc 57.55 ± 2.02
8% Dried mushroom	b54.16 ± 3.01	c 94.20 ± 8.17	b73.6 ± 9.73	c-36.53 ± 22.17	a93.83 ± 5.57	d6.49 ± 3.60	ed 0.68 ± 2.75	a-96.50 ±13.68	b0.28 ± 1.65	a 6.79 ± 1.78	b13.76 ± 1.48	Dc54.42 ± 5.84
P<	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001		
	***	***	***	***	***	***	***	***	***	***		

BPC: Beef patty control fortified with 0% dried mushroom. BP1: Beef patty fortified with 4% dried mushroom. BP2: Beef patty fortified with 8% dried mushroom. BP3: Beef patty fortified with 12% dried mushroom. HCH: hypercholesterolemic. Each value is the mean ± SD, n: 5/ group. (a, b, c, d, e, and f): means in the same column with different superscript differ significantly at p< 0.05. *P< 0.05. **P< 0.01. *** P< 0.001. ****P< 0.0 001.