

Full Length Research Paper

Protective effects of mushroom and their ethyl extract on aging compared with L-carnitine

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The effects of 10% dried mushroom and 300 mg mushroom extract on the lipid profile, lipid peroxidation and liver functions of rats were evaluated and compared with L-carnitine. Food intake, body weight and histological examination of liver tissues were also evaluated. Supplementation diet of rats with 10% dried mushroom, 300 mg mushroom extract and 300 and 600 mg L-carnitine resulted in a significant decrease in total lipids, triglycerides, total cholesterol, low-density lipoprotein, very low-density lipoprotein, aspartate amino transferase (AST) enzyme, alanine amino transferase (ALT) enzyme, alkaline phosphatase (AP) enzyme, malonaldehyde (MAD) and body weight compared to control. However, glutathione peroxidase enzyme (GSH) and food intake were significantly increased in rats supplemented with 10% dried mushroom, 300 mg mushroom extract and 300 and 600 mg L-carnitine. Liver tissues of rats were improved by the supplementation with 10% dried mushroom and 300 mg L-carnitine. However, the supplementation with 300 mg mushroom extract and 600 mg L-carnitine were more effective in improving the liver tissues. These results suggest that mushroom and their extract can improve the antioxidant status during ageing and minimize the occurrence of age-associated disorders associated with involvement of free radicals.

Key words: Dried mushroom, mushroom extract, L-carnitine, lipids profile, liver function, lipid peroxidation.

INTRODUCTION

Aging is associated with biochemical and structural alterations which are thought to result in motor and cognitive impairments and in increased susceptibility to neurodegenerative diseases (Freo et al., 2002; Terry and Buccafusco, 2003). The free radical theory of aging proposed that aging is due to the accumulation of unrepaired damage from free radical attack on cellular components. Modern thinking theory proposes that aging is caused by a shift in the balance between the pro-oxidative and anti-oxidative processes in the direction of

the pro-oxidative state (Harman, 1992; Beckman and Ames, 1998; Cadenas and Davies, 2000). Mushroom had high amounts of proteins, carbohydrates and fibers with low fat contents (Bárbara et al., 2008). Furthermore, mushroom had significant levels of vitamins, namely thiamine, riboflavin, ascorbic acid and vitamin D2, as well as minerals (Mattila et al., 2000). Regarding their medicinal value, mushroom is effective as antitumor, antibacterial, antiviral and hematological agents and in immunomodulating treatments (Wasser and Weis, 1999; Yang et al., 2002). Mushroom species had been shown to possess antioxidant capacity in *in-vitro* systems (Ribeiro et al., 2006). Seline and Johein (2007) reported that the L-carnitine concentration in mushroom ranged from 133 - 530 mg/kg DM (mean 320 mg/kg DM). The free L-carnitine concentration in mushroom ranged between 73 - 383 mg/kg DM (mean 218 mg/kg DM), which represented $65 \pm 8\%$ of total carnitine content. L-carnitine, a nutrient normally synthesized from methionine

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Abbreviations: **AST**, Aspartate amino transferase; **ALT**, alanine amino transferase; **AP**, alkaline phosphatase; **MAD**, malonaldehyde; **GSH**, glutathione peroxidase.

and lysine in the liver and kidney. L-carnitine to produce energy. L-carnitine deficiency decreases LCFA availability for oxidation, thereby resulting in LCFA accumulation in the cytosol, and decreased ketone and energy production. Other L-carnitine functions include the maintenance of adequate free coenzyme-A required for various metabolic pathways, the protection of cells against toxic accumulation of acyl-coenzyme-A compounds by shuttling acyl groups out of the mitochondria, and the storage and transport of energy (Catherine et al., 2006). Also, L-carnitine supports the immune system and enhances the antioxidant system (Bremer, 1997). The objectives of this study were to evaluate the effects of dried mushroom and mushroom extract on the lipids profile, lipid peroxidation and liver functions of aging rats as compared with L-carnitine.

MATERIALS AND METHODS

The mushroom *P. ostreatus* was purchased from the Food Technology Research Institute, Agricultural Research Center. L-carnitine was purchased from Sigma Chemical Co. (St. Louis, MO, USA). Total cholesterol, HDL-cholesterol, LDL-cholesterol, total lipids, alkaline phosphatase (AP), aspartate amino transferase (AST), alanine amino transferase (ALT), glutathione peroxidase (GSH) and malonaldehyde (MDA) kits were obtained from Randox Laboratories Ltd, England.

Preparation of the dried mushroom and mushroom extract

The mushroom *P. ostreatus* was dried in the shade and then finely powdered. Dried mushroom were ground to pass through a 60 mesh sieve. Fifty grams of dried mushroom were extracted with 150 ml of 95% ethanol using a Soxhlet apparatus. The residue was filtered and concentrated to a dry mass by vacuum distillation and used as mushroom extract.

Experimental animal

Male albino rats of Wister strain weighing approximately 310 ± 6 g were used. A total of thirty Albinos male rats were raised in the animal house of Food Technology Research, Agriculture Research Center, Giza, Egypt. The animals were fed with a basal diet for 7 days as an adaptation period. The basal diet was formulated according to AIN (1993) and consisted of casein (12%), corn oil (10%), cellulose (5%), salt mixture (4%), vitamin mixture (1%) and starch (68%); Water was available *ad libitum*. The animals were divided to five groups; the first group (control) was fed with the basal diet. The second and the third groups were fed with L-carnitine (300 and 600 mg/kg body weight/day, respectively), in 0.89% saline at physiological pH. The fourth and fifth groups were fed with 10% dried mushroom and 300 mg/kg body weight/day mushroom extract, respectively. Blood samples were taken at the start and the end of experiment (4 weeks). The blood samples were obtained from orbital plexus veins by means of fine capillary glass tubes according to the method described by Schermer (1967). The blood samples were placed in dry and clean centrifuge tubes and allowed to clot for 1 - 2 h at room temperature. Serum was removed using a Pasteur pipette and centrifuged for 20 min at 1100 x g. The clean supernatant serum was kept frozen until analysis. Body

weights of animals were recorded at the start and the end of experiment.

Measurements of biochemical variables

The serum triglycerides, low density lipoprotein (LDL), high-density lipoprotein (HDL), total cholesterol and total lipids were determined according to the methods described by Fossati and Prencipe (1982); Friedwald et al. (1972); Demacker et al. (1980); Richmond (1973); Frings and Dunn (1979), respectively. Alanine amino transferase (ALT), aspartate amino transferase (AST) and alkaline phosphatase (AP) enzymes were measured according to the methods described by Bergmeyer and Harder (1986); Kachmar and Moss (1976); Varley et al. (1980), respectively. Glutathione peroxidase enzyme (GSH) and malonaldehyde (MDA) were determined according to the methods described by Hu (1994); Jentsch et al. (1996).

Histopathology examinations

Small specimens of the organs liver were taken from each experimental group, fixed in neutral buffered formalin, dehydrated in ascending concentration of ethanol (70, 80 and 90%), cleared in xylene and embedded in paraffin. Sections of 4 - 6 μ m thickness were prepared and stained with hematoxylin and eosin according to Bancroft et al. (1996).

Statistical analysis

The results recorded as the mean \pm SD of seven replicates. The experimental data were subjected to an analysis of variance (ANOVA) for a completely randomized design using the Statistical Analysis System (SAS, 2000). Duncan's (1995) multiple range tests were used to determine the differences among means at the level of 5%.

RESULTS AND DISCUSSION

Effect of dried mushroom, mushroom extract and L-carnitine on lipids profile of aging rats

Data shown in Table 1 showed the effect of 10% dried mushroom, 300 mg mushroom extract and L-carnitine on total lipids, triglycerides and total cholesterol. Total lipids was significantly ($p \leq 0.05$) reduced in rats supplemented with mushroom and L-carnitine. The reduction in the total lipids was ranged between 6.85 - 13.79%. There was no significant ($p > 0.05$) difference in total lipids between rats supplemented with 300 mg L-carnitine and those supplemented with 10% dried mushroom. Rats supplemented with 600 mg L-carnitine had a higher ($p \leq 0.05$) total lipids than those supplemented with 300 mg mushroom extract.

Supplementation of rats diet with mushroom and L-carnitine resulted in a significant ($p \leq 0.05$) decrease in triglycerides and total cholesterol. Triglycerides were reduced by 30.47 - 40.92%. However, total cholesterol was reduced by 16.38 - 27.0%. Supplementation of rats diet with 300 mg mushroom extract and 600 mg

Table 1. Effect of dried mushroom (DM), mushroom extract (ME) and L-carnitine (LC) on lipid profile of aging rats.

Groups	Total lipids			Triglycerides			Total cholesterol		
	0 week	4 weeks	% Changes	0 week	4 weeks	% Changes	0 week	4 weeks	% Changes
Aged rats control	482.64 ± 9.34	475.05 ± 4.88 ^a	-1.57	197.6 ± 10.16	165.32 ± 13.18 ^a	-16.33	165.28 ± 4.68	158.74 ± 4.37 ^a	-3.95
Aged rats with 300 mg LC	480.23 ± 4.98	443.11 ± 6.96 ^b	-8.45	202.5 ± 8.71	140.78 ± 3.93 ^b	-30.47	161.51 ± 3.81	135.05 ^b ± 4.67 ^b	-16.38
Aged rats with 600 mg LC	479.65 ± 5.57	425.73 ± 6.25 ^c	-11.24	195.85 ± 9.64	121.55 ± 5.25 ^c	-37.93	163.25 ± 3.69	119.16 ± 3.82 ^c	-27.00
Aged rats with 10% DM	475.60 ± 8.23	442.95 ± 6.4 ^b	-6.85	205.30 ± 11.21	135.46 ± 4.44 ^b	-34.0	162.60 ± 4.02	131.47 ± 5.77 ^b	-19.13
Aged rats with 300 mg ME	480.05 ± 7.54	413.82 ± 2.39 ^d	-13.79	202.70 ± 6.34	119.73 ± 1.47 ^c	-40.92	160.52 ± 4.53	120.00 ± 3.65 ^c	-25.24
LSD	10.16	7.87	-	11.34	12.12	-	5.34	7.76	-

Values are means ± SD of 6 rats from each group. Means in the same column with different superscripts are significantly different ($p \leq 0.05$).

L-carnitine were more ($p \leq 0.05$) effective in reducing triglycerides and total cholesterol than those supplemented with 10% dried mushroom and 300 mg L-carnitine. On the other hand, supplementation of rat's diet with 300 mg mushroom extract and 600 mg L-carnitine were similar ($p > 0.05$) in reducing triglycerides and total cholesterol. Supplementation of rat's diet with 10% dried mushroom and 300 mg L-carnitine were also similar ($p > 0.05$) in reducing triglycerides and total cholesterol.

Bobek et al. (1994) found that rats fed with semisynthetic diet containing 0.3% cholesterol and supplemented with 5% dried whole oyster mushroom had reduced serum and liver cholesterol levels by 32 and 55%, respectively. Panchamoorthy and Carani (2007) reported that treated rats with L-carnitine caused a significant reduced in TG as compared to untreated rats. L-carnitine is known to promote the transport of cytosolic long-chain fatty acids into the mitochondrial matrix for β -oxidation, thereby providing mitochondrial energy (Diaz et al., 2000; Eskandari et al., 2004). L-carnitine may lower plasma TG by increasing the utilization and/or oxidation of fatty acids for energy or possibly by

altering very low-density lipoprotein synthesis (Tanaka et al., 2004).

Effect of dried mushroom, mushroom extract and L-carnitine on lipoprotein of aging rats

Data shown in Table 2 showed that the high density lipoprotein in rats was not affected ($p > 0.05$) by the supplementation with 10% dried mushroom and 300 mg L-carnitine. However, rats supplemented with 300 mg mushroom extract and 600 mg L-carnitine had a higher ($p \leq 0.05$) value of high density lipoprotein than those of the control. High density lipoprotein was increased in these rats by 23.7 - 29.09%. Low density lipoprotein was ($p \leq 0.05$) reduced in rats supplemented with mushroom and L-carnitine by 29.22 - 54.39%. Supplementation rats with 300 mg mushroom extract and 600 mg L-carnitine were more ($p \leq 0.05$) effective in reducing low density lipoprotein than those supplemented with 10% dried mushroom and 300 mg L-carnitine. On the other hand, supplementation of rat's diet with 300 mg mushroom extract and 600 mg L-carnitine were similar ($p > 0.05$) in reducing low density

lipoprotein. Supplementation of rat's diet with 10% dried mushroom and 300 mg L-carnitine were also similar ($p > 0.05$) in reducing low density lipoprotein.

Very low density lipoprotein in rats was ($p \leq 0.05$) reduced by the supplementation with mushroom and L-carnitine. Very low density lipoprotein was reduced in these rats by 30.44 - 40.09%. Supplementation of rats diet with 300 mg mushroom extract and 600 mg L-carnitine were more ($p \leq 0.05$) effective in reducing very low density lipoprotein than those supplemented with 10% dried mushroom and 300 mg L-carnitine. Supplemented with 300 mg mushroom extract and 600 mg L-carnitine was not significantly ($p > 0.05$) differ in their effect on very low density lipo-protein. Also, no significant ($p > 0.05$) difference was found in very low density lipoprotein between rats supplemented with 10% dried mushroom and those supplemented with 300 mg L-carnitine. These results are in agreement with those reported by Sidereal and Volgin (1996); Lofgren et al. (2005) they found that L-carnitine stabilizes the level of lipids peroxidation decreases concentration of total lipids, triglycerides, total cholesterol, phospholipids and lipoproteins of low and very low

Table 2. Effect of dried mushroom (DM), mushroom extract (ME) and L-carnitine (LC) on lipoprotein of aging rats.

Groups	High density lipoproteins			Low density lipoproteins			Very low density lipoproteins		
	0 week	4 weeks	% changes	0 week	4 weeks	% changes	0 week	4 weeks	% changes
Aged rats control	49.18 ± 2.56	53.31 ± 4.07 ^C	+8.40	76.58 ± 4.83	72.37 ± 3.03 ^a	-5.49	39.52 ± 2.54	33.06 ± 2.63 ^a	-16.34
Aged rats with 300 mg LC	48.36 ± 3.14	55.46 ± 5.44 ^{bc}	+14.68	72.65 ± 3.59	51.42 ± 0.37 ^b	-29.22	40.50 ± 1.93	28.17 ± 0.78 ^b	-30.44
Aged rats with 600 mg LC	49.00 ± 3.51	60.61 ± 3.04 ^{ab}	+23.70	75.08 ± 4.69	34.24 ± 0.13 ^C	-54.39	39.17 ± 2.31	24.31 ± 1.05 ^C	-37.93
Aged rats with 10% DM	47.65 ± 2.76	52.73 ± 3.61 ^C	+10.66	73.89 ± 4.72	51.65 ± 3.10 ^b	-30.09	41.06 ± 1.49	27.09 ± 0.88 ^b	-34.02
Aged rats with 300mg ME	48.53 ± 3.85	62.65 ± 2.97 ^a	+29.09	71.45 ± 5.88	33.40 ± 1.18 ^C	-53.25	40.54 ± 1.67	23.95 ± 1.55 ^C	-40.09
LSD	4.06	6.92	--	6.28	3.69	--	2.81	2.55	--

Values are means ±SD of 6 rats from each group. Means in the same column with different superscripts are significantly different ($p \leq 0.05$).

Table 3. Effect of dried mushroom (DM), mushroom extract (ME) and L-carnitine (LC) on liver functions of aging rats.

Groups	Aspartate aminotransferase (AST)			Alanine aminotransferase (ALT)			Alkaline phosphatase (AP)		
	0 week	4 weeks	% changes	0 week	4 weeks	% changes	0 week	4 weeks	% changes
Aged rats control	82.65 ± 2.15	76.85 ± 2.47 ^a	-7.02	42.67 ± 1.26	40.21 ± 3.45 ^a	-5.76	62.01 ± 2.35	62.42 ± 2.46 ^a	+0.66
Aged rats with 300 mg LC	81.26 ± 3.46	62.57 ± 3.71 ^b	-23.00	43.52 ± 1.99	34.46 ± 3.31 ^b	-20.82	61.53 ± 1.98	48.50 ± 4.95 ^b	-21.17
Aged rats with 600 mg LC	80.39 ± 3.19	45.13 ± 1.63 ^d	-43.86	41.75 ± 2.39	26.97 ± 1.59 ^C	-35.40	62.37 ± 3.05	32.12 ± 2.14 ^d	-48.5
Aged rats with 10% DM	83.07 ± 2.57	51.69 ± 2.73 ^C	-37.78	41.98 ± 2.96	27.05 ± 1.28 ^C	-35.57	63.40 ± 2.98	51.00 ± 2.82 ^b	-19.55
Aged rats with 300mg ME	82.45 ± 2.31	49.16 ± 2.95 ^{cd}	-40.33	42.16 ± 3.07	23.24 ± 2.07 ^C	-44.88	60.90 ± 3.44	41.72 ± 1.30 ^C	-31.4
LSD	4.16	5.30	--	3.54	4.26	--	3.92	4.89	--

Values are means ±SD of 6 rats from each group. Means in the same column with different superscripts are significantly different ($p \leq 0.05$).

density.

Effect of dried mushroom, mushroom extract and L-carnitine on liver functions of aging rats

Data in Table 3 indicated that, the aspartate amino transferase enzyme (AST) in rats was significantly ($p \leq 0.05$) reduced by the supplementation with mushroom and L-carnitine. Mushroom reduced AST enzyme by 37.78 - 40.33%. However, L-carnitine reduced it by 23 - 43.86%. Rats supplemented with 300 mg L-

carnitine had a higher ($p \leq 0.05$) AST enzyme than those supplemented with mushroom and 600 mg L-carnitine. Those supplemented with 300 mg mushroom extract and 600 mg L-carnitine were not significantly ($p > 0.05$) differed in their effect on AST enzyme.

Supplemented rats with mushroom and L-carnitine had a lower ($p \leq 0.05$) alanine amino transferase enzyme (ALT) than that of the control. Mushroom and L-carnitine reduced ALT enzyme by 35.57 - 44.88% and 20.82 - 35.40%, respectively. Supplemented rats with 10% dried mushroom, 300 mg mushroom extract and 600

mg L-carnitine were more effective ($p > 0.05$) in reducing ALT enzyme than those supplemented with 300 mg L-carnitine. No significant ($p > 0.05$) difference was found in ALT enzyme among rats supplemented with 10% dried mushroom, 300 mg mushroom extract and those supplemented with 600 mg L-carnitine. The alkaline phosphatase enzyme (AP) in rats was significantly ($p \leq 0.05$) reduced by the supplementation with mushroom and L-carnitine. Mushroom reduced AP enzyme by 19.55 - 31.4%, however, L-carnitine reduced it by 21.17 - 48.5%. Supplemented rats with 300 mg L-carnitine had a higher ($p \leq 0.05$) AP enzyme

Table 4. Effect of dried mushroom (DM), mushroom extract (ME) and L-carnitine (LC) on malonaldehyde (MAD) and glutathione peroxidase enzyme (GSH) of aging rats.

Groups	MDA (n mol/ml)			GSH (U/ml)		
	0 week	4 weeks	% changes	0 week	4 weeks	% changes
Aged rats control	24.87 ± 2.15	25.32 ± 1.47 ^a	+1.80	11.0 ± 3.56	11.51 ± 2.70 ^d	+1.64
Aged rats with 300 mg LC	26.37 ± 2.11	19.01 ± 0.53 ^c	-27.9	13.21 ± 3.41	21.86 ± 2.68 ^c	+59.1
Aged rats with 600 mg LC	25.61 ± 1.68	16.98 ± 1.39 ^d	-33.69	12.14 ± 2.94	31.49 ± 2.97 ^a	+128.6
Aged rats with 10% DM	23.98 ± 2.52	21.48 ± 1.55 ^b	-10.4	10.28 ± 4.62	19.61 ± 1.67 ^c	+57.23
Aged rats with 300mg ME	24.45 ± 1.46	18.15 ± 0.90 ^{cd}	-25.7	14.05 ± 4.09	26.03 ± 2.52 ^b	+79.23
LSD	2.65	2.05		4.76	4.44	

Values are means ±SD of 3 rats from each group. Means in the same column with different superscripts are significantly different ($p \leq 0.05$).

than those supplemented with 600 mg L-carnitine. Supplemented rats with 10% dried mushroom had a higher ($p \leq 0.05$) AP enzyme than those supplemented with 300 mg mushroom extract. Supplemented rats with 10% dried mushroom and 300 mg L-carnitine were not significantly ($p > 0.05$) differed in their effect on AP enzyme.

L-carnitine and mushroom restores the changes of ALT, AST and AP activities due to their antioxidant effects and their ability to act as a radical scavenger, thereby protecting membrane permeability. Augustyniak and Skrzydlewska (2009) found that ALT and AST after ethanol intoxication of their activity increased by about 80%. L-carnitine partly prevented these changes. It was manifested by a statistically significant decrease in the activity of ALT and AST, by about 20% in comparison with the ethanol group.

Effect of dried mushroom, mushroom extract and L-carnitine on MDA and GSH in aging rats

Data shown in Table 4 indicate that, the MDA was ($p \leq 0.05$) reduced by 10.4 - 33.69% in rats supplemented with mushroom and L-carnitine. Supplementation of rats diet with 300 mg mushroom extract and 600 mg L-carnitine were more ($p \leq 0.05$) effective in reducing MDA than those supplemented with 10% dried mushroom and 300 mg L-carnitine. On the other hand, supplementation of rats with 300 mg mushroom extract and 600 mg L-carnitine were similar ($p > 0.05$) in reducing MDA. Supplementation of rats with 300 mg mushroom extract and 300 mg L-carnitine were also similar ($p > 0.05$) in reducing MDA. Rats supplemented with 10% dried mushroom had higher ($p \leq 0.05$) MDA than those supplemented with 300 mg L-carnitine. Augustyniak and Skrzydlewska (2009) found that administration of L-carnitine to rats intoxicated with ethanol significantly modifications in the serum and liver. The level of MDA was decreased by about 30% in the blood serum in protects lipids and proteins against oxidative comparison

to the ethanol group. Glutathione is a major, non-protein thiol in living organisms which performs a key role in co-ordinating the innate antioxidant defense mechanisms. It is involved in the maintenance of the normal structure and function of cells, probably by its redox and detoxification reactions (Gueeri, 1995).

The GSH in rats was significantly ($p \leq 0.05$) increased by the supplementation with mushroom and L-carnitine. Mushroom increased GSH by 57.23 - 79.23%. However, L-carnitine increased it by 59.1 - 128.6%. Supplemented rats with 300 mg L-carnitine and 10% dried mushroom had a lower ($p \leq 0.05$) GSH than those supplemented with 600 mg L-carnitine and 300 mg mushroom extract. Supplemented rats with 10% dried mushroom and 300 mg L-carnitine were not significantly ($p > 0.05$) differed in their effect on GSH. Supplementation of rats diet with 600 mg L-carnitine was more ($p \leq 0.05$) effective in increasing GSH than those supplemented with 300 mg L-carnitine, 10% dried mushroom and 300 mg mushroom extract. Augustyniak and Skrzydlewska (2009) found that L-carnitine caused a significant increase in the liver and blood serum GSH level by 25%. An increase in the levels of GSH in aged rats treated with mushroom extract as a source of antioxidant has also been reported by Jayakumar et al. (2006).

Effect of dried mushroom, mushroom extract and L-carnitine on food intake and body weight of aging rats

Table 5 showed the effect of dried mushroom, mushroom extract and L-carnitine on food intake and body weight of rats. Either L-carnitine or mushroom significantly ($p \leq 0.05$) increased food intake and reduced body weight in rats. There was no significant ($p > 0.05$) difference in food intake between rats supplemented with L-carnitine and mushroom. Supplementation of rats with L-carnitine was more ($p \leq 0.05$) effective in reducing body weight than those supplemented with mushroom. Supplemented rats with 300 mg L-carnitine and 600 mg L-carnitine were not

Table 5. Effect of dried mushroom (DM), mushroom extract (ME) and L-carnitine (LC) on food intake and body weight of aging rats.

Groups	Food intake	Body weight	
		Initial	final
Aged rats control	9.3 ± 1.14 ^b	312.5 ± 9.84	328.4 ± 8.59 ^a
Aged rats with 300 mg LC	17.9 ± 2.13 ^a	313.7 ± 8.37	275.4 ± 8.14 ^c
Aged rats with 600 mg LC	18.1 ± 1.98 ^a	310.8 ± 9.15	267.9 ± 7.62 ^c
Aged rats with 10% DM	17.3 ± 1.85 ^a	312.7 ± 9.46	316.3 ± 8.04 ^b
Aged rats with 300mg ME	17.5 ± 1.90 ^a	316.9 ± 8.53	312.5 ± 8.16 ^b
LSD	2.35	7.54	8.95

Values are means ±SD of 6 rats from each group. Means in the same column with different superscripts are significantly different ($p \leq 0.05$).

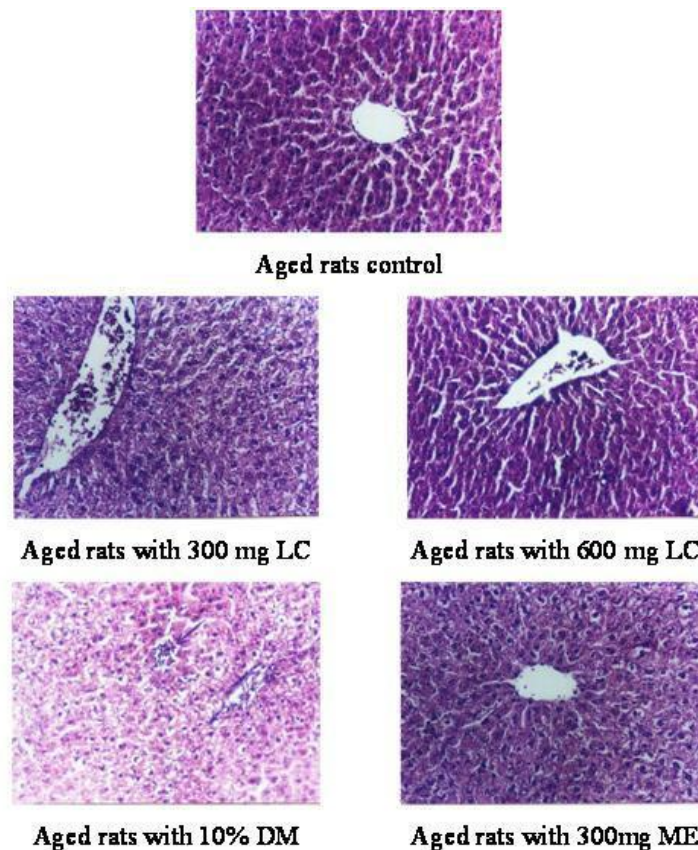


Figure 1. Effect of dried mushroom (DM), mushroom extract (ME) and L-carnitine (LC) on histological examination of liver tissues of aging rats.

significantly ($p > 0.05$) differed in their effect on body weight. Similar effect was observed in rats supplemented with 10% dried mushroom and 300 mg mushroom extract. The rationale for carnitine supplementation as a weight-loss agent is based on the assumption that regular oral ingestion of the substance increases its intra-cellular concentration. This would trigger the increased of fat oxidation and gradual reduction of the body's fat reserves (Barnett et al., 1994 and Villani et al., 2000).

Histopathological examinations

Figure 1 showed the histological examination of liver tissues of aged rats fed with basal diet. The examination of rats liver tissues showed congestion of the central vein and infiltration with chronic inflammatory cells. The changes in the liver tissues of aged rats fed with basal diet and supplemented with 300 mg L-carnitine and 10% dried mushroom are showed slight hydropic degeneration

of hepatocytes and vacuolations of some hepatocytes and small focal hepatic necrosis. The examination of liver tissues for rats fed with basal diet which was supplemented by 600 mg L-carnitine and 300 mg mushroom extract indicated apparent normal hepatocytes. These results agree with those reported by Jayakumar et al. (2006). From the above results, it could be concluded that mushroom and their extract were comparable to L-carnitine in controlling lipids oxidation. Dried mushroom and their extract can improve the antioxidant status during ageing and minimize the occurrence of age-associated disorders associated with involvement of free radicals.

REFERENCES

- AIN (1993). American Institute of Nutrition (AIN), Purified diet for laboratory rodent. Final Report. ADHOC Writing Diet. J. Nutr., 123: 1939-1951.
- Augustyniak A, Skrzydlewska E (2009). L-Carnitine in the lipid and protein protection against ethanol-induced oxidative stress. Alcohol. 43: 217-223.
- Bancroft D, Steven A, Turner R (1996). Theory and Practice of Histological Techniques, 4th Churchill Livingstone, Edinburgh, London, Melbourne.
- Bárbara R, Rosário L, Paula B, Rosa M, Rui F, Paula B, Inês Q, Patrícia V (2008). Comparative study of phytochemicals and antioxidant potential of wild edible mushroom caps and stipes, Food. Chem., 110: 47–56.
- Barnett C, Costill DL, Vukovich MD (1994). Effect of L-carnitine supplementation on muscle and blood carnitine content and lactate accumulation during high intensity sprint cycling. Int. J. Sport Nutr., 4: 280-286.
- Beckman B, Ames BN (1998). The free radical theory of aging matures. Physiol. Rev., 78:547-581.
- Bergmeyer HU, Harder M (1986). A colorimetric method of the determination of serum glutamic oxaloacetic and glutamic pyruvic transaminase, Clin. Biochem., 24: 481-486.
- Bobek P, Ozdın L, Kuniak L (1994). Mechanism of hypocholesterolemic effect of oyster mushroom (*Pleurotus ostreatus*) in rats: reduction of cholesterol absorption and increase of plasma cholesterol removal. Z. Ernährungswiss., 33: 44–50.
- Bremer J (1997). The role of carnitine in cell metabolism. In: De Simone C, Famularo, G. (Eds.), Molecular Biology Intelligence Unit Carnitine Today (Landes Bioscience Austin, TX, USA, International Copyright). Springer Verlag, Heidelberg, Germany. pp. 4-37.
- Cadenas E, Davies KJ (2000). Mitochondrial free radical generation, oxidative stress and aging, Free Radical. Biol. Med., 29: 222–230.
- Catherine MC, Michael CS, Michael LC, Charles TH, Bruce J, Richard A (2006). Carnitine supplementation in premature neonates: Effect on plasma and red blood cell total carnitine concentrations, nutrition parameters and morbidity, Clin. Nutr., 25: 886–896.
- Demacker PM, Von-Janssen HE, Hifman AM, Vant's Lear A, Jansen AP (1980). Measurement of high density lipoprotein cholesterol in serum. Comparison of six isolation methods combined with enzymatic cholesterol analysis. Clin. Chem., 26: 1780-1789.
- Diaz M, LopezF, Hernandez F, Urbina JA (2000). L-carnitine effects on chemical composition of plasma lipoproteins of rabbits fed with normal and high cholesterol diets, Lipids. 35: 627–632.
- Duncan DB (1995). Multiple range and multiple F test Biometrics 11: 1-42.
- Eskandari HG, Burak Cimen MY, Lulufer T, Arzu K, Ugur A (2004). Short term effects of l-carnitine on serum lipids in STZ-induced diabetic rats Diabetes. Res. Clin. Pract., 66: 129–132.
- Fossati P, Prencipe I (1982). Serum triglycerides determination colorimetrically with an enzyme that produce hydrogen peroxide, Clin. Chem., 28: 2077-2083.
- Freo U, Pizzolato G, Dam M, Ori C, Battistin L (2002). A short review of cognitive and functional neuroimaging studies of cholinergic drugs: implications for therapeutic potentials, J. Neural. Transm., 109: 857–870.
- Friedwald WT, Levy RT, Fredrickson DS (1972). Estimation of the concentration of low density lipoprotein cholesterol in plasma without use of the preparative ultracentrifuge, Clin. Chem., 8: 499-505.
- Frings CS, Dunn RT (1979). Colorimetric method for determination total serum lipids based on the sulphopospho vanillin reaction, Am. J. Clin. Pathol., 53: 89-91.
- Gueeri H (1995). Influence on prolonged ethanol intake on the level and turnover of alcohol and aldehyde dehydrogenase and glutathione. Adv. Exp. Med. Biol., 23: 133–134.
- Harman D (1992). Free radical theory of aging. Mutation Res., 275: 257-266.
- Hu ML (1994). Measurement of protein thiol groups and glutathione in plasma. Methods. Enzymol., 233: 380-385.
- Jayakumar T, Ramesh E, Geraldine P (2006). Antioxidant activity of the oyster mushroom, *Pleurotus ostreatus*, on CCl4-induced liver injury in rats. Food Chem. Toxicol., 44: 1989–1996.
- Jentezch AM; Bachmann H, Furst P, Biesalski HK (1996). Improved analysis of malonaldehyde in human body fluids, Free Radic. Biol. Med., 20: 251-260.
- Kachmar JF, Moss DW (1976). Enzymes, In: Philadelphia PA. W.B. Saunders Co. (edited by Tiez N). Fundamentals Clin. Chem., pp. 666-672,
- Lofgren I, Zern T, Herron K, West K, Sharman M, Volek J, Shachter N, and Fernandez M (2005). Weight loss associated with reduced intake of carbohydrate reduces the atherogenicity of LDL in premenopausal women. Metabolism. 54 (9):1133-1141.
- Mattila P, Suonpaa K, Piironen V (2000). Functional properties of edible mushroom, Nut., 16: 694–696.
- Panchamoorthy R, Carani V (2007). Fructose-induced hepatic gluconeogenesis: Effect of L-carnitine. Life Sci., 80: 1176–1183.
- Ribeiro B, Rangel J, Valentão P, Baptista P, Seabra R, Andrade P (2006). Contents of carboxylic acids and two phenolics and antioxidant activity of dried Portuguese wild edible mushrooms. J. Agric. Food. Chem., 54: 8530–8537.
- Richmound W (1973). Preparation and properties of cholesterol oxidase from *Nocardia* sp. and its application to enzymatic assay of total cholesterol in serum, Clin. Chem., 19: 1350.
- SAS (2000). Statistics analysis system. SAS Users Guide: Statistics Version 5th Ed., SAS. Institute Inc., Cary N.C.
- Schermer S (1967). The Philadelphia: F. A. Davies Co. Blood Morphology of Laboratory Animals. p. 359.
- Seline KG, Johein H (2007). The determination of L-carnitine in several food samples, Food. Chem., 105: 793-804.
- Sidereal NG, Volgin DV (1996). Effect of L-carnitine on lipid peroxidation and lipid composition in blood serum in hemic hypoxia. Ukr Biokhim Zh; 68 (5): 54–58.
- Tanaka Y, Sasaki R, Fukui F, Waki H, Kawabata T, Okazaki M (2004). Acetyl-l-carnitine supplementation restores decreased tissue carnitine levels and impaired lipid metabolism in aged rats. J. Lipid Res., 45: 729-735.
- Terry J, Buccafusco JJ (2003). The cholinergic hypothesis of age and Alzheimer's disease-related cognitive deficits: recent challenges and their implications for novel drug development. J. Pharmacol. Exp. Ther., 306: 821–827.
- Varley H, Gewenlock A, Bell M (1980). London; Williams Heinemen Medical books, Ltd., Pract. Clin. Biochem., 1(5): 741-897
- Villani R, G Gannon J, Self M, Rich PA (2000). L-carnitine supplementation combined with aerobic training does not promote weight loss in moderately obese women, Int. J. Sport. Nutr. Exerc. Metab., 10: 199-206.
- Wasser SP, Weis AL (1999). Medicinal properties of substances occurring in higher Basidiomycetes mushrooms: Current perspective (review). International J. Med. Mushrooms, 1: 31–62.
- Yang JH, Lin HC, Mau JL (2002). Antioxidant properties of several commercial mushrooms, Food Chem., 77: 229–235.