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Level of Liver Lipid Profile as Influenced by *Agaricus bisporus* in Experimental Hypercholesterolemia

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Abstract

Coronary Artery Disease has been identified as the major cause of death in India. A primary risk factor is hypercholesterolemia. This experiment was carried out to determine the long-term hypolipemic effect of mushroom (*Agaricus bisporus*) in wistar albino rats with high fat diet induced hypercholesterolemia. Male albino rats of wistar strain with an initial body weight of about 265-275 gm were used for the study. The animals were divided into four groups consisting of six rats per group. Group I, Control animals, Group II, Mushroom treated rats, Group III, High fat diet and Group IV, HFD and 5% dry mushroom powder fed rats. Results showed that, the dietary hypercholesterolemia caused by the high fat diet was improved by mushroom (MR) feeding, accompanied by a significant decrease in the liver lipid profile level. The liver lipid profiles were increased in high fat diet animals and were also corrected to near normal by mushroom treatment. Histological evaluation of the animals revealed centrolobular degeneration of hepatocytes, small and large drop fatty degeneration of the liver. Considering the results of the histological studies on liver it is evident that the treatment with mushroom (MR) fed rats expresses their individual remedial impact. Therefore, the objective of relevance of using mushrooms in diet proved to be very effective in bringing down the liver lipid levels in experimental animals.

Key words: *Agaricus bisporus*, high fat diet, histopathological slides mushroom and liver lipids.

Introduction

Cardiovascular disease is the most common causes of death in the developed and developing countries of the world. (Cannon,2007). India is expected to have the largest population of the elderly by 2001. One-fifth of Indian population is projected to be above 60 years by 2020 AD, with a life expectancy close to 70 years. Besides, the incidence and prevalence of coronary heart disease is on the increase. Although the economic, social and health burden of coronary heart disease in elderly and old persons are well-known, as the older adults are probably unstructured, poorly co-ordinated, often inappropriate, and hence, in great need of re-organization.

Coronary Artery Disease has been identified as the major cause of death in India. A primary risk factor is hypercholesterolemia, which contributes to the hardening of arteries. Hypercholesterolemia is a major risk factor for atherosclerosis (Wissler, 1992). The cellular events occurring during the progression of lesions in hypercholesterolemic animals are almost exactly mirrored by those observed in human atherosclerotic coronary arteries in hearts removed during transplant operations (Ross, 1993). Hypercholesterolemia is also related to diabetes and it has role in inducing oxidative stress .(Bhat nagar and Soran ,2008). Oxidative stress is thought to contribute to the development of a wide range of diseases including Alzheimer's disease, Parkinson's disease , neuro degeneration, aging and most importantly cancer (Khan , 2010).

High incidence of hypercolesterolemias and unfavorable development of cardiovascular mortality makes the search for natural substances with hypolipemic activity and their further investigation attractive.(Pavel Bobek et al ., 1993) Hypercholesterolemia is an important primary risk factor in the development of coronary heart disease.

Clinical studies have demonstrated the therapeutic importance of correcting the hypercholesterolemic effect and many indigenous plants have gained importance in the prevention and treatment of coronary heart disease, but they require systematic and scientific evaluation. India stands amidst a demographic transition having different age groups, showing increasing non-communicable diseases including coronary heart disease which reveals an interesting trend.

Recent reports have shown that mushroom are good for diet and have a significant hypolipidemic effect. Since the ancient times , mushrooms have been regarded as important food items which have preventive and protective effects against many disorders including hyper-cholesterolemia. In humans, 50% or more of the total cholesterol is derived from de novo synthesis (Steinberg, 1989). The initial step in lowering cholesterol is with a special diet low in fat and saturated fatty acids but rich in crude fibers. The best known organisms for potential producers of bisporus from edible higher Basidiomycetes mushrooms are species of the genus Agaricus. The role of dietary fiber in lipid metabolism and atherogenesis has been extensively studied (Vahouny, 1982; and Story, 1985). However, the focus has been on fiber of vegetable, fruit and cereal origin (Anderson, 1987, Kuske and Feldman, 1987).

Mushrooms because of their high fiber content, plant sterols and proteins, microelements and low calorie content

are almost ideal for a nutrition program aimed at the prevention of caidiovascular diseases (Pavel Bobek et al., 1991). Including more mushrooms as part of natural hypocholesterolemic antisclerotic diets was suggested first in oriental medicine (Sun et al., 1984.). Wood – rotting shiitake mushroom (Lentinus edodes) effectively lowered plasma cholesterol (C) levels in laboratory animals (Kurusawa et al.,1982.) and humans (Suzuki and Oshimas. 1976.).The active substance with hypocholesterolemic effects was isolated and identified as a derivative of adenine (Rokujo et al .,1970.).

The population of the elderly is increasing rapidly in India and they are more vulnerable to coronary heart diseases, this provoked the investigation on the role of edible mushroom Agaricus bisporus on hypercholesterolemia. Therefore, in this study, the objective was to determine the long-term hypolipemic effect of mushroom (*Agaricus bisporus*) in wistar albino rats with high fat diet induced hypercholesterolemia.

Materials and Methods

Description of the study area

This research was carried out at Taramani campus ,Institute of Basic Medical Sciences ,Chennai-600113, which is located in Taramani about a distance of 15 km from Anna nagar,Chennai.(capital city of Tamil nadu,South India).

Experimental material

Male albino rats of wistar strain with an initial body weight of about 265-275 gm were used for this study. They were obtained from Fredrick Institute of Plant Protection and Toxicology, (Fippat) Padappai, 601301, Chengai District, Tamil Nadu, India. After the arrival from the breeding farm, they were left to adopt in the animal room having free access to tap water and standard laboratory feed supplied by Hindustan Lever Limited, Bombay, marketed under the trade name "Gold mohur feeds".

Experimental design and procedure

The animals were divided into four groups consisting of six rats per group. Group I: Control animals. These animals served as control to get the base line data on biochemical parameters. GroupII: Mushroom treated rats only (MR). These animals received mushroom powder 5% by oral feeding (Fruit bodies and Stipes 3:1 ratio). This 5% was based on the 5gm mushroom powder and 95gm normal feed. Group III: High fat Diet (HFD). This group consists of hypercholesterolemic rats in which hypercholesterolemia was induced by feeding to the animals a diet referred to as atherogenic diet (atherodiet). This diet was based on the formula of Hahn et al., (1977) and it was prepared by mixing the commercial

pelleted feed with the following ingredients; Cholesterol – 5%, Sucrose – 20%, Hydrogenated Vegetable Oil-20%, Lactose-2%, Choline Chloride-0.4%, Sodium Cholate-0.2%, 2-Thiouracil-0.15%, (47.75%) remaining normal pelleted feed-52.25%, Group IV: HFD and 5% dry mushroom powder, fed rats (47.75% + 5.0% + 47.25%) as above. Each group was identified by a specific marking on different parts of the body (group I rats were marked on the face, group II on the head, Groups III on the neck while group IV were marked on the abdomen). The different groups were subjected to the experimentation (Yearul Kabir et al., 1987.). The animal in each group were tested in batches so that biochemical studies could be carried out in a phased manner. The animals sacrificed on a day were not from the same group but from different groups.

The feed was pulverised and mixed with sugars, choline chloride, bile salt and thiouracil. Hydrogenated fat was melted separately and cholesterol was dissolved in hot fat. The fat was then poured on the dried feed mixer prepared earlier and mixed well into the form of dough. This dough was separated into 20g ratio in warm condition. The animals were provided with 20g each of this diet which was daily being replenished. The control groups II and treated groups IV feed were administered with 5% mushrooms. Similar treatment was conducted with experimental hypertensive rats.

Mushroom (*Agaricus bisporus*)

Mushroom has two main components namely pileus and stipes. The mushroom diet was prepared by mixing the .components in the ratio of 3:1 for the reasons that pileus is made up of high protein and low carbohydrate concentration and the stipes have more minerals (Poongkodi and Sakthisekaran 1995.) About 1kg of fresh mushroom when dried gives 60gms to 80gms of dry mushroom powder. The mushroom at the rate of 5% was given to the rats, then the dosage had already been standardised as described by Bobek et al., (1993).

Preparation of tissue homogenate

Liver was dissected out. The liver tissue was washed with ice cold saline, and 10% homogenate of the washed tissues were prepared in 0.1M Tris-Hcl buffer pH 7.4. Lipids were extracted from the liver according to the method of Folch et al., (1957).The cholesterol in tissue extract was estimated by the method of Parekh and Jung (1970). Triglyceride in tissue extract was estimated by the method of Rice (1970). Phospholipids were estimated by the method of Rouser et al., (1970) after digesting the lipid extract with perchloric acid (1959). Assay of free fatty acid was carried out according to the method of Chromy et al., (1977.) with minor

modification. Instead of using 2, thiazolylazo- 4-methoxy phenol the color was developed using 0.1 percent diethyl dithiocarbamate following the procedure of Regouw et al., (1977). The free cholesterol was precipitated as its digitonide by the method of Mookerjee and Sadhu (1955) Which was a modification of the procedure of Sperry and Web (1950) and cholesterol was estimated with precipitate by the method of Leffler and Douglad.(1963) .The esterified cholesterol was arrived at from the difference between the total cholesterol and free cholesterol levels.

Histopathology studies

Soon after killing the animals, a portion of the tissue liver were kept in formalin for 2 weeks for fixing the tissues. The tissues were washed by running tap water, dehydrated in the descending grades of isopropanol and finally cleaned in xylene. Then the tissues were embedded in molten paraffin wax. Sections were cut out at 10µm thickness, stained with haematoxylin and eosin. The sections were then viewed under polarized photo microscope to observe any histopathological changes in the tissues.

Statistical analysis

Data were treated statistically using student's t-test. Where there was a significant difference between groups, the comparison of groups was made by turkey's multiple range tests at 0.05 level of significance.

Results

The results of the administration of feeding mush room on level of liver lipids of hypercholesterolemic rats are presented in table 1.

Table 1 Level of liver lipid profile in control and experimental rats

Investigation mg/g tissue	Group I C	Group II C+MR	Group III HFD	Group IV HFD+MR
Total lipid	142.80±3.81	130.55±3.18	231.75±3.18	184.30±4.38
Total cholesterol	20.60±2.40	22.30±1.70	50.30±2.93	30.57±4.98
Triglyceride	42.45±0.78	34.70±3.25	73.00±2.83	54.95±0.07
Phospholipid	69.60±1.13	62.50±3.54	98.40±1.56	76.35±0.64
Free fatty acid	0.24±0.01	0.96±0.04	7.03±0.03	3.53±0.08
Free Cholesterol	19.05±0.21	19.10±1.13	23.70±3.25	20.70±2.40
Ester Cholesterol	11.15±0.35	9.97±0.80	41.47±2.07	21.96±0.64

Values are expressed as mg/gm tissue and Mean ±S.D for six animals in each group.

The results as shown in table 1 indicated that the administration of feeding mushroom (MR) on level of liver lipids of hypercholesterolemic rats, and the administration of high fat diet (HFD) for 105 days, increased

liver total lipid, cholesterol, triglyceride, phospholipid, free cholesterol, ester cholesterol except free fatty acid (FFA), from 142mg to 231mg/dl, 20mg to 50mg/dl, 42mg to 73mg/dl, 69mg to 98mg/dl, 19mg to 23mg/dl, 11mg to 41mg/dl respectively. Group IV animals showed a reduction in total lipid, Cholesterol, triglyceride, phospholipid, free cholesterol and ester cholesterol except free fatty acid, the decreased being greater in 5% MR treated groups. The decrease in total lipid, cholesterol, triglyceride, phospholipid, free cholesterol and ester cholesterol level observed in MR treated groups (Gr IV) could be mainly due to the increase in free fatty acid level observed in these animals. Free cholesterol level does not show marked variation. Treatment of normal animals with 5% MR slightly altered total lipid, cholesterol, triglyceride, phospholipid, free fatty acid, free cholesterol and ester cholesterol level in liver (table 1).

Hypercholesterolemic rats showed increased level of cholesterol, triglyceride, and phospholipid in liver but free fatty acid, free cholesterol and ester cholesterol were decreased in liver. The concentration of total lipid, total cholesterol, triglyceride, phospholipid, free fatty acid, free cholesterol and ester cholesterol were influenced by the dietary cholesterol level whereas that of all lipid profile level tended to decrease gradually in response to the 5% dietary mushroom level (Gr. IV) except free fatty acid. The magnitude of increment became significant at all hepatic lipid profile levels except free cholesterol. Early dietary manipulation did not affect the plasma lipid profiles (data not shown). At the end of the 15th week, the liver lipid profile levels increased markedly (Gr. III) except free fatty acid.

Liver tissue

The liver of control animals consists of 7 lobes. All lobes were reddish in color (Fig 1). The liver of mushroom treated rats appears to be healthy after a period of 105 days (Fig 2). The normal liver lobes of rats treated with high fat diet (HFD) changed to a pale color and hepatocytes were observed all over the surface of the liver as well as the inter view which showed a reddish white due to the accumulation of fat globules. For the high fat diet plus mushroom treated rats, the liver still appears healthy but with a reduction in the fat accumulated.

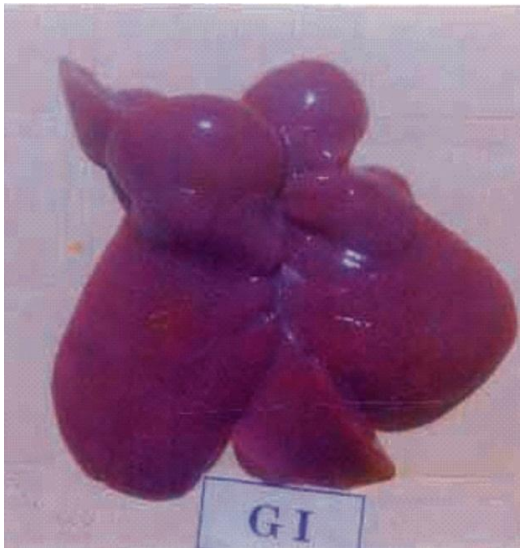

Figure1. Group I: Liver of control

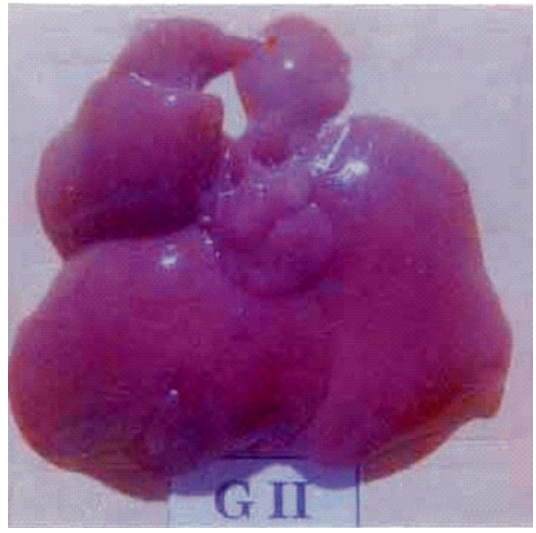

Figure 2. Group II:Control+MR treated rats

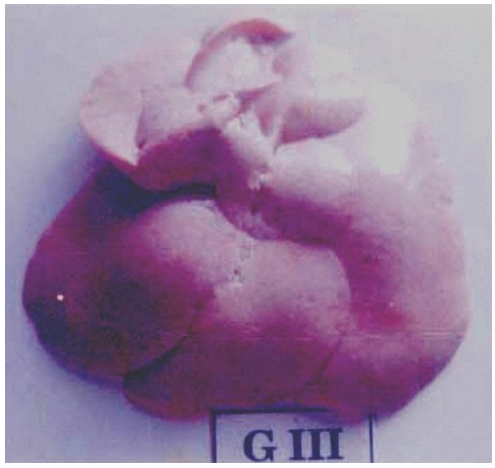

Figure 3. Group III: HFD

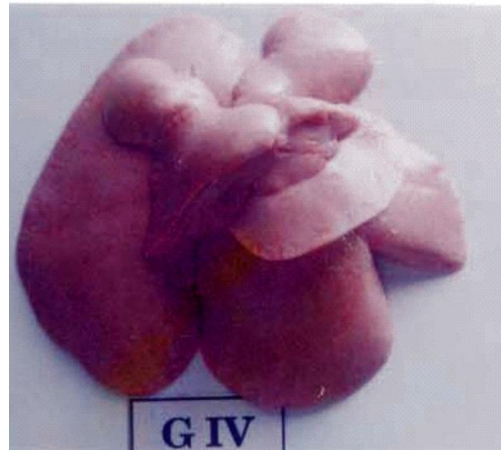

Figure 4. Group IV: HFD & MR treated animals.

The polarized electron microscope preparations indicated that the cell surface of the hepatic cells from control group was smooth, with large spherical nucleus and nucleoli showing a fibrilo-grannular network structure. The cytoplasm showed a granular appearance. There were profuse amount of rough endoplasmic reticulum especially around the nuclear envelope and between the mitochondria. The endothelial cells were extremely thin with an electron-lucent cytoplasm. The kupffer cells were macrophages lining the sinusoids, with the endothelial cells (Figure 5).

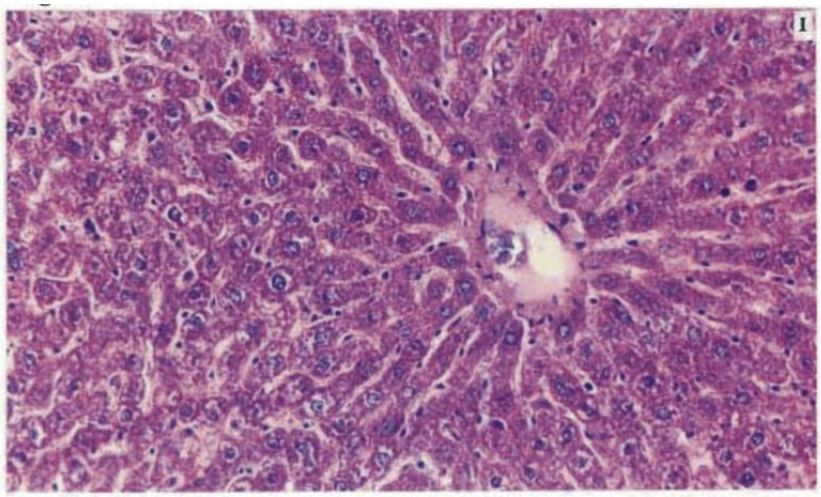

Figure 5. Section of normal rat liver showing hepatocytes and a portal tract with branches of the hepatic artery, portal vein and interlobular bile duct without any signs of vascular changes (H+E staining, 320x)

In animals treated with MR, the hepatic cell surfaces showed lateral inter digitations. The nuclei were found to contain a distinct nucleolus. The cytoplasm of the hepatic cells contained a fairly large amount of rough and smooth endoplasmic reticulum with many round mitochondria. The hepatic sinusoids were found to contain only one discontinuous layer of lining cells. The endothelial cells were extremely thin with an electron lucent cytoplasm and contained different types of vacuoles. At the end of 105 days treatment showed normal histopathological features without any sign of toxicity.

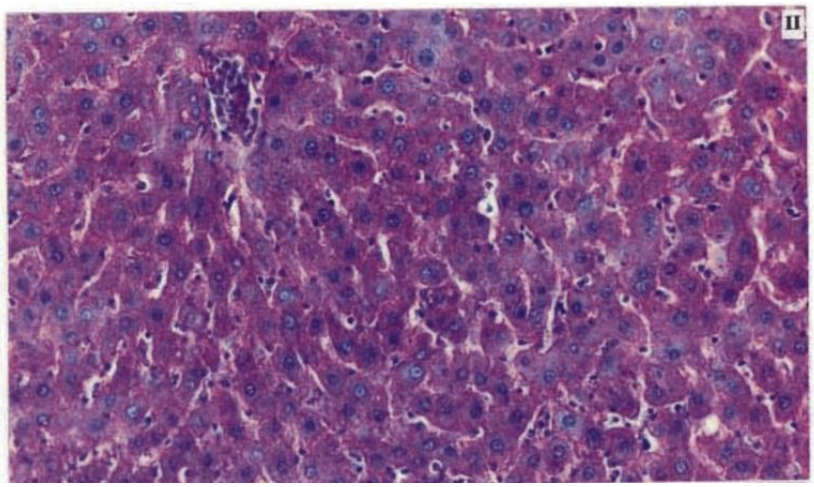

Figure 6. Group II 5% MR treated animals showing section of liver fed on normal diet (H+E Staining, 320 x)

The histopathological analysis of group III animals fed with high fat diet (HFD) is presented in Fig 7a and 7b. The result of HFD on the liver revealed signs of changes. The changes were significant ($P< 0.05$) in comparison with control (groupI) and HFD + MR treated (group IV) animals. It shows a mild vascular congestion in the central vein and moderate congested red blood cells in the sinusoids, nuclear changes and centrilobular necrosis. In hypercholesterolemic condition, there is vacuolation of liver cells suggestive of probable fatty change which is very prominent. Focal areas of necrosis with round cells in filtration are seen near the portal system and hydropic change. (H&E, Staining 320x).

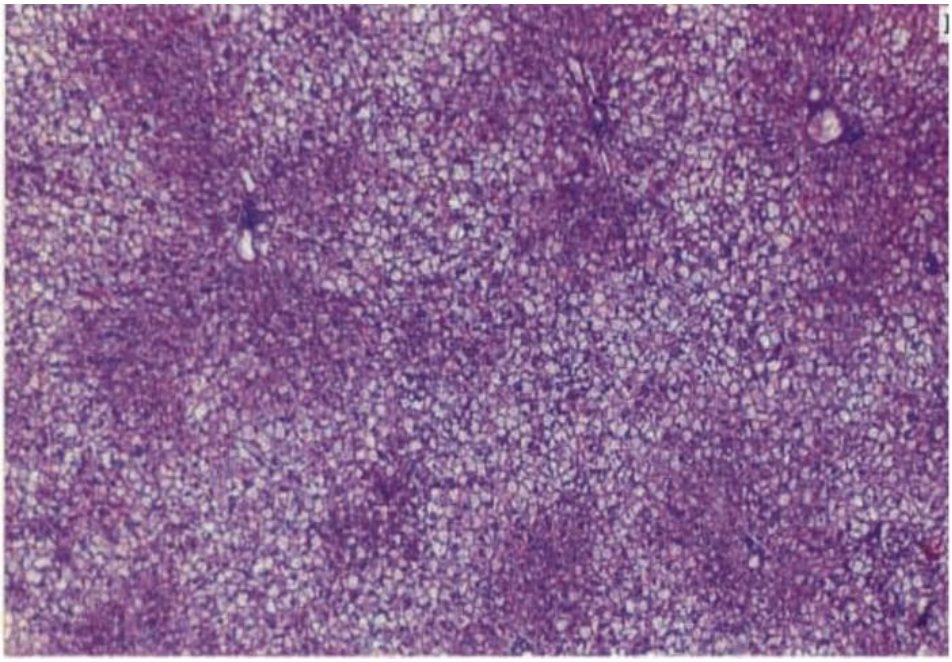

Figure 7a. Group III (HFD) Section of liver showing small vacuolation (H+E, Staining 80x)

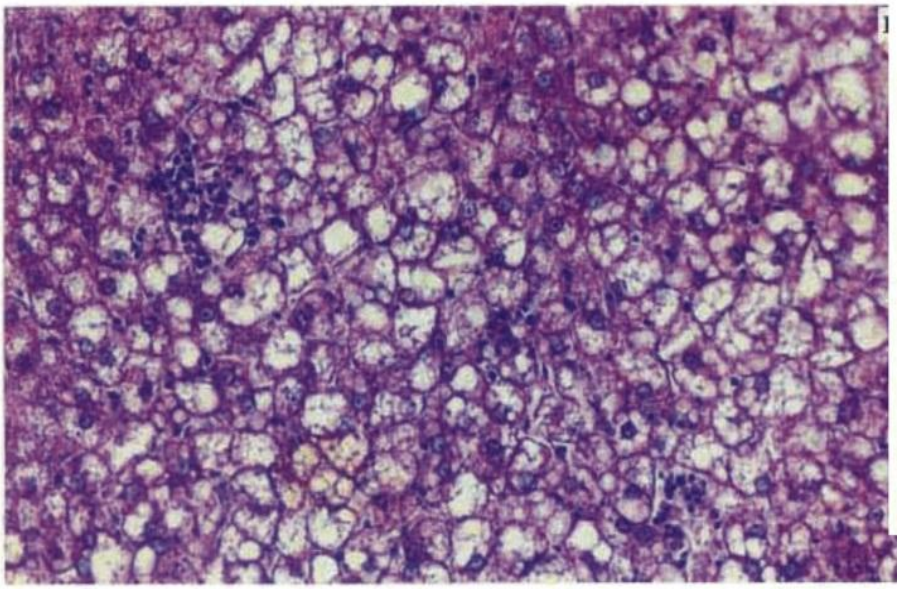

Figure 7b. Section of liver showing big vacuolation, vascular congestion and fatty change in liver parenchyma (H&E, Staining 320x).

In the 5% MR treated animals, a focal collection of round cells in liver parenchyma and fatty change were pronounced. Moreover, moderate to severe vascular changes and sinusoidal congestion as well as red blood cells accumulation around the draining path with extensive necrosis was observed (Fig 8a and 8b)

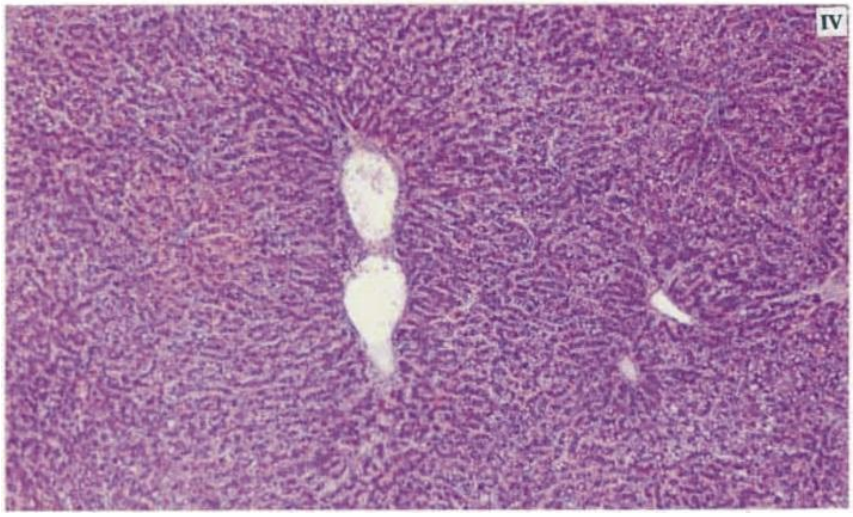

Figure 8a. HFD + 5% mushroom treated animals; Section showing focal collection of round cells in liver parenchyma (H+E staining, 80x)

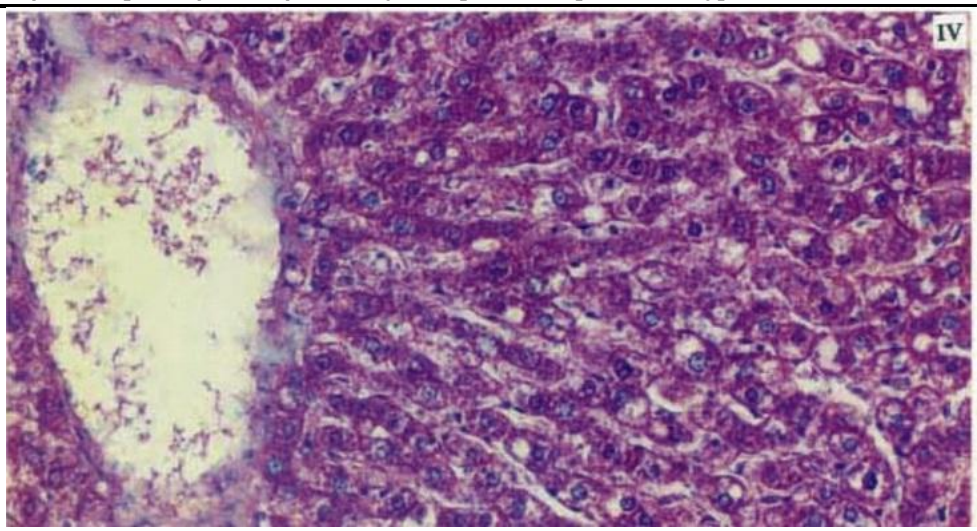

Fig 8b. Section showing focal areas of necrosis and pronounced fatty Changes (H+E, Staining, 320x)

Discussion

Oyster mushroom contains a number of substances with potential effects on the absorption of cholesterol or other lipids. Particularly the water soluble gel-forming components of the fiber matter (beta-1,3 D-glucan with a low degree of polymerization, forming 15-20% of dry mass) can interact with bile acids and affect the formation of micelles. In this way such substances could interfere with the absorption of cholesterol (Bobek *et al*., 1994). The mushroom sterols (0.2% of dry matter) can reduce cholesterol absorption by competitive inhibition (Ikeda *et al*., 1988). Other substances present in oyster mushroom (lignin and pectin-2 and 6% dry matter (Story. 1985), undigested protein residues (Sugano *et al*., 1988), chitin (5% of dry matter) transformed in the gastrointestinal tract probably to chitosan (Zemek *et al*., 1987.) can increase the excretion of bile acids by the ability to bind them. Increased excretion can in turn reduce the pool of bile acids in liver and enhance the cholesterol catabolism to bile acids in liver (Havel. 1988). This could be the reason for the result in the present study. The increase degradation of cholesterol in the liver as observed in the lower cholesterol accumulation in the organ of animals fed the mushroom containing diet was 39.3% in group IV ($p<0.05$). A reduced absorption of cholesterol together with enhanced degradation of cholesterol can significantly contribute to the decrease of cholesterol content in serum and liver (Bobek *et al*., 1993). Another possibility that cannot be excluded is that the decrease of liver cholesterol releases the block of APo B/E receptors leading to a stimulation of plasma cholesterol removal demonstrated in hamsters (Bobek *et al*., 1993). Hypolipidemic effect of oyster mushroom by the ethanol-

stirmulated (Bobek *et al* .,1991.) or streptozotocine diabetes-stimulated over-production of VLDLis propably mediated by the enhanced plasma VLDL removal(Bobek et al 1993.). Bobek et al., (1993) had reported that oyster mushroom decreased the cholesterol concentration in serum and in liver, not only in highly hypercholesterolemic rats (high cholesterol intake), but also in rats with mild hypercholesterolemia fed a diet containing 0.009% of cholesterol. This corroborates the findings in this study. In the case of low cholesterol diet the accelerated plasma removal of VLDL, LDL, and HDL in the hypocholesterolemic effect of oyster mushroom can play a more important role than the affected absorption (Bobek *et al*., 1993). In oyster mushroom Iwai (1974) was not able either to confirm or to exclude the presence of eritadenin, a substance with high hypocholesterolemic activity from a related fungus Lentinus edodes. The effect of this substance is explained by the inhibition of the formation of primary lipoproteins with no effect on lipid absorption.

Other studies reveal elevation of phospholipids without change in the ratio of cholesterol to phospholipid has been reported in hypothyroidism (Peters and Mann .1943).Total phospholipid levels were either normal or increased (Ching Tong Liu. 1965). Liver phospholipid level was increased with hypercholesterolemic rats, but after the administration of mushroom, liver phospholipid was decreased effectively. Therefore, liver cholesterol, triglyceride, phosphor-lipid, free cholesterol and ester cholesterol increased dose dependently with dietary cholesterol. This effect was evident at the dietary cholesterol level of 5% and the magnitude of the increase in the liver was more marked in triglyceride, phospholipid and cholesterol. This agrees with the findings of Huang *et al* ., **(**1986).

Beside, in this study, elevated triglyceride levels were observed in hypercholesterolemic liver tissue. Administration of MR however lowers the triglyceride level. Keys *et al*., (1979) had reported an increased triglyceride formation in liver of animals administered with mushroom.and propylthiourcil treated rats.

Administration of mush room elevates free fatty acid concentration. It was obvious that the reducing effect produced by mushroom on liver lipid was dose dependent. Dietary cholesterol has been shown to reduce fatty acid oxidation, which in turn, increases the levels of hepatic and plasma triglycerol (Fungwe. *et al*., 1993). Hepatic triglyceride and cholesterol (free, esterified and total) levels were therefore affected by the fat and protein composition of the diets.

The histopathological evaluation revealed centrolobular degeneration of hepatocytes, small and large drop fatty degeneration of the liver. The degree and range of these changes were larger in rats fed with mushroom and high fat diet fed rats than control diet rats. The liver of rat treated with HFD showed an extensive cytoplasmic vacuolation, hydropic degeneration. At ultrastructural level, the cytoplasm of the hepatocytes in this study showed different sizes of vacuoles. The cytoplasmic vacuolization implies increased permeability of cell membranes, leading to increased intracellular water (Wilhelm, 2007). Also the hepatic cytoplasm of rats administered with HFD displayed increased number of mitochondria, ballooning of the mitochondrial cristae (Shimizu *et al*., 1996.).

The most striking feature of rats treated with HFD + MR was the appearance of a large number of mitochondria and ample ER around the nucleus. The potential hepatoprotective role of ush room may be associated with its antioxidant constituents such as selenium, tocopherol and Vitamin C. Mush room has been shown to be effective against free radicals ((Filipek and Misik 1993) and induced cellular transformation. Considering the results of the histological studies on liver, it is evident that the treatment with MR on HFD fed rats expresses their individual remedial impact.

Conclusion

This study confirmed pronounced hypercholesterolemic (or commonly hypolipemic) and heptoprotective efficiency of oyster mushroom in experimental hyperlipoproteinemias induced by diet. When considering the low content of the whole mushroom in the diet, its profound and long-term hypolipemic effect was surprising. Therefore, the results represent an experimental basis of perspective exploitation of mushroom in the prevention of hypercholesterolemia. The results suggest that *Agaricus bisporus* could successfully enhance the effectiveness of hypolipemic diets considering the results of the histological studies on liver. It was evident that the treatment with MR on HFD fed rats expresses their individual remedial impact. This area of work which dealt with the dietary supplementation with mushroom was hitherto unexplored and needs further attention. If mushroom therapy proves useful in rodents as this study indicates, it could be extrapolated to human cases and portends a brighter future for coronary heart disease victims and hundreds of lives could be saved every year.

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