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Effect of Agaricusbisporus on the Lipid Peroxidation, CK and LDH of Hypercholesterolemic Rats

G.K.Poongkodi[1],G.P.Harshitha Priya[2] and G.P.Harithra Priya[2]

[1]Department of Biochemistry, Dr. ALM P.G. Institute of Basic Medical Sciences, University of Madras, India.
[2]GRT MahalakshmiVidyalaya Matriculation Higher Secondary School, Chennai-83.Tamil Nadu.

Correspondence author: Department of Chemistry, College of Natural and Computational Studies ,Ambo University, Ethiopia: Email:gkpoongkodi@gmail.com

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Abstract: Mushrooms are the manifestation of a common saying, medicines and foods have a common origin in constituting both the nutritionally functional food and a source of physiologically beneficial medicine. The present studies were undertaken to investigate the effect of mushroom, Agaricusbisporus on the lipid peroxidation, CK and LDH of HFD induced hypercholesterolemic rats for 15 weeks. Male albinorats of wistar strain with an initial body weight of about 265-275gm were used for this study. The animals were divided into four groups consisting of six rats per group. Group I,control fed the basal diet. The Group II, control and MR, the Group III, high fat diet (HFD) and the Group IV, HFD and MR fed animals. At the end of the fifteenth week, blood collection from the sinus venosus, plasma was separated. Aorta and heart were dissected out and lipid peroxidation, CK and LDH were determined according to the well established methodology. Regarding lipid peroxidation, the level in plasma, aorta and heart were grossly elevated in hypercholesterolemic rats. Administration of MR marginally decreased in the level of MDA in hypercholesterolemic rats. The creative kinase and lactate dehydrogenase were increased significantly in plasma and decreased in heart on atherodiet feeding. (Animals).Dietary restriction induced a significant reduction in the enzyme level.

Keywords: Agaricusbisporus, Creatine Kinase, High Fat diet, Lactate dehydrogenase, Lipid Peroxidation, and Mushroom.

Abbrevations: CK: Creatine Kinase,HC: Hypercholesterolemia; HFD: High Fat diet; LDH: Lactate dehydrogenase;MR: Mushroom;and MI: Myocardial Infraction.

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1. Introduction:

The objective of the study is to investigate the effect of Mushroom (MR) on the activities of plasma ,aorta and heart MDA levels in albino rats and the study also deals with the characteristics of creatine kinase ,lactate dehydrogenase in plasma and heart of albino rats.

Higher Basiodiomycetes mushroom have been used in folk medicine throughout the world since ancient times [1]. Mushrooms are the manifestation of a common saying, 'Medicines and Foods have a common origin', in constituting both a nutritionally function food and a source of physiologically beneficial medicine. Many Centuries ago, medicinal properties of mushrooms have been recognized in China, Korea and Japan. Although from ancient times, mushrooms have been treated as a special kind of nutraceutical, they have received a remarkable interest in recent decades. Major medicinal properties attributed to mushrooms include anticancer activity, antibiotic activity, immune response- stimulating effects, anti-hypersensitive and blood lipid lowering and antiperoxidative effects[2,3].

Hypercholesterolemia (HC) is a major risk factor for atherosclerosis and coronary heart disease[4]. It is characterized by coronary endothelial dysfunction the hallmark of which Is an altered vasodilators[5].It may also promote is chemic tissue damage by enhancing the vulnerability of the microcirculation to the deleterious effects of ischemia and other inflammatory stimuli

(strokes). This leads to increase in the incidence of myocardial ischemia and cardiac events[6]. Atherosclerosis is accompanied with the production of free radicals by endothelial and vascular smooth muscles[7]. Hypercholesterolemic state leads to an increase radical production and thereby elevates lipid peroxides[8].

The pathological role of free radicals in the development of many important diseases, e.g. atherosclerosis of cancer has been proved beyond doubt [11]. Polluted environment and unhealthy lifestyles lead to greater exposure of humans to the negative influence of free radicals. The sufficient level of antioxidants in the body is the only effective defensive mechanism. Many of non-traditional medical preparations like mushrooms contain substances with antioxidant activity[12]. Creative Kinase (CK) is a cytoplasmic enzyme composed of M and or B subunits that associate to form CK - MM, CK -MB and CK -BB. The CK - MB iso-enzyme has to be an important tool for diagnostic evaluation of acute coronary syndromes. CK - MB'S Characteristic rise and fall in serial measurements is nearly pathognomonic for establishing the diagnosis of MI [13]. Lactate dehydrogenase (LDH) are measured in plasma to assist in the diagnosis of myocardial infarction. However, an increased plasma CK value and chest pain suggestive of ischemic heart diseases are also common in patients with primary hypothyroidism. Because ischemic heart disease and primary hypothyroidism are both the relatively common clinical presentations. It is important for clinical chemists to monitor carefully the diagnostic interpretation placed on "cardiac enzyme" [14]. Therefore the present investigation has been undertaken to explore the antiperoxidative, CK and LDH effect of the mushroom in HFD induced rats in a dose dependent manner of hypercholesterolemia. Accordingly, in hypercholesterolemic rats, we evaluated the effect of ingesting MR on lipid peroxidation ,CK and LDH levels.

2. Materials and Methods:

Chemicals: Cholesterol, Choline chloride, Sodium cholate,2-thio uracil were purchased from sigma chemicals (St.Louis ,USA).TBA, MDA, NAD and DNP and all the other chemicals and reagents used were of analytical grade and were obtained from SD fine chemicals, Mumbi, India.

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".[4]

The standard rat pelletised feed was used for the preparation of the rat diet comprising of 100g of the diet contained 21g protein, 5g lipid, 4g crude fiber, 8g ash, 1g calcium, 0.6g phosphorus and 55g nitrogen – free extract carrying 360 kcals of metabolisable energy. In addition to the above mentioned items, the feeds were enriched by stabilized Vitamins A, D_3, Vitamin E, Vitamin K, Vitamin B_{12}, thiamine, riboflavin, pantothenic acid, niacin, Choline chloride, folic acid, all minerals and trace elements. It contained 100-500µg of copper, 3-5µg of iron and 90-100µg of zinc.

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. Group II: 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 the following diet, to be referred to as atherogenic diet (atherodiet) was fed to the animals. This diet was based on the formula of Hahn *et al.,* [15] and it was prepared by mixing the commercial pelleted feed with the ingredients listed below:- 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; For example, group I rats were marked on the face, group II on the head, Groups III on the neck Group IV on the abdomen. Then different groups were subjected to the experimentation. The animal in each of group were tested, not all at time but 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 the hot

fat. The fat was poured on the dried feed mixer prepared earlier and mixed well into the form of dough. This dough was separated into 20g ratios still in condition warm. The animals were provided with 20g for each by this diet. The diet was replenished daily. The control groups II and treated groups IV feed were administered with 5% mushrooms. Similar treatment was conducted with experimental hypertensive rats.

2.1. Mushroom (*Agaricusbisporus*):

Mushroom has two main components namely pileus and stipes. The diet was prepared by mixing the .above components in the ratio of 3:1 for the reasons that pileus is formed of high protein and low carbohydrate concentration and the stipes have more of minerals [16]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 standardized [17, 18].

2.2. Preparation of Tissue Homogenate

At the end of the fifteenth week, the rats were anaesthetised by ether inhalation and cardiac puncture subsequent to blood collection from the sinus venosus as whole blood sample with EDTD as the anticoagulant and centrifuged for 10min for separation of plasma. Aorta and heart were dissected out and washed with ice cold saline, and 10% homogenate of the washed tissues were prepared in 0.1M Tris - HCl buffer PH 7.4.The homogenates were kept in the cold room (4^{o}C) and assays were made on the same day.

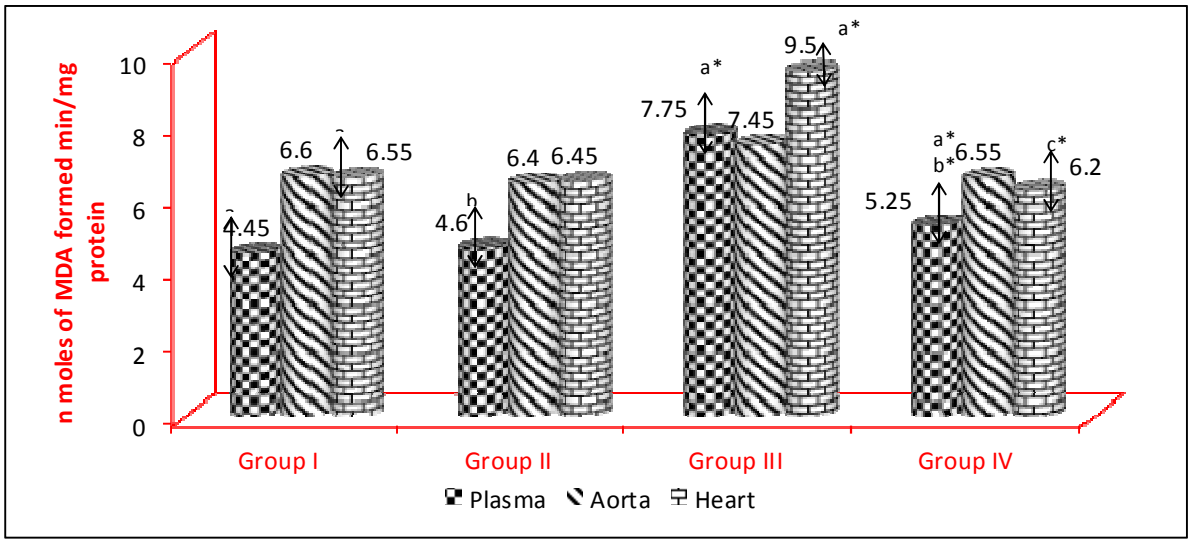
The level of plasma lipid peroxides was determined by the method of Yagi [19]. Tissue lipid peroxidation was measured by the method of Devasagayam [20]. Plasma and heart creatine kinase activity was determined by the method of Okinaka *et al.*, [21]. The method adopted for the estimation of plasma and heart LDH was that of King [22] respectively.

2.3. Statistical Methods

The data obtained were analyzed using the one way analysis of variance [ANOVA] to determine differences [23] and Tukey's Multiple Range Test [TMRT] to separate the means ±S.D. The P value <0.05 was considered to be statistically significant.

3. Results

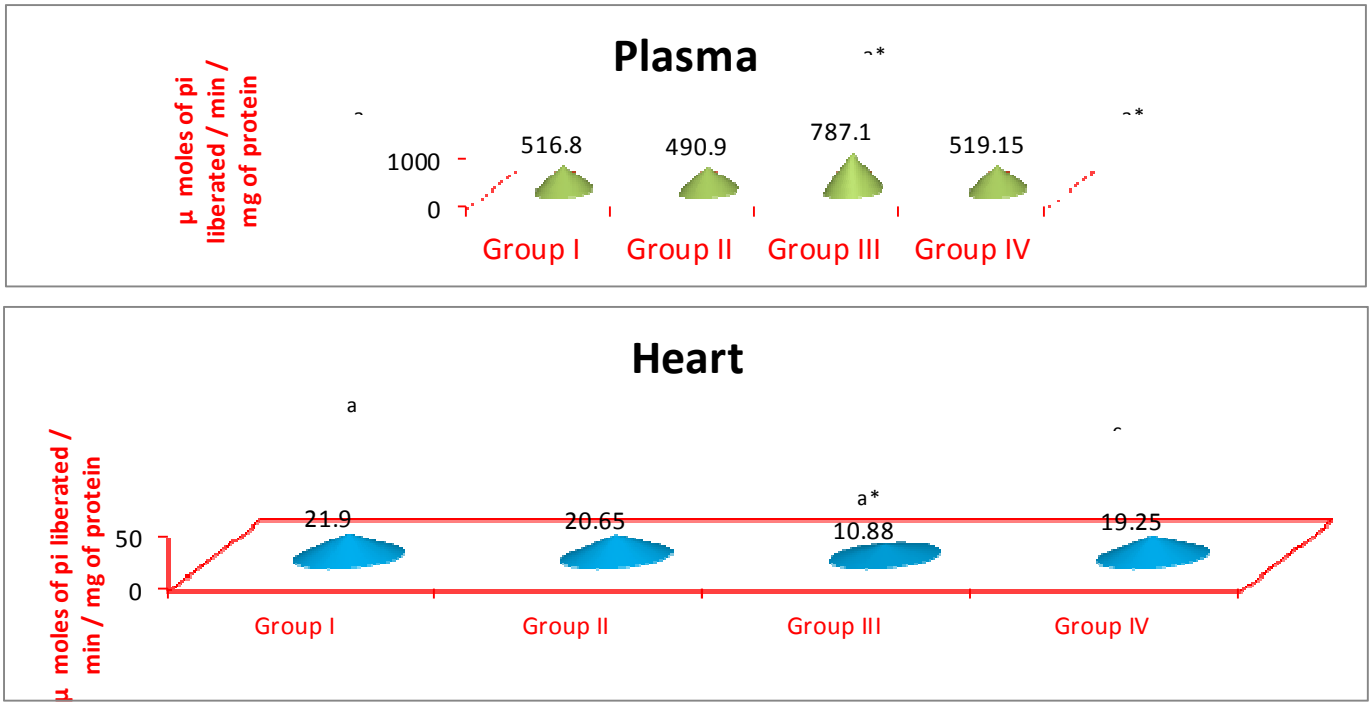
Fig 1.Level of lipid peroxidation in control and experimental rats



Values are expressed as mean ± SD for six animals in each group.
a =denotes the comparison of Gr III, IV, with I; b=Gr IV with II; C= Gr IV with III; statistically significant alterations are expressed as *P<0.05.

In Fig (1) the plasma ,aorta, heart the MDA level (P<0.05)got reduced by(Gr.IV5.25±0.21,6.55±0.49, 6.20±0.28) in wistar albino rats supplemented with diet containing 5% mushroom(Gr.IV) is compared with HFD diet animals(Gr.III7.75±0.07,7.45±0.35, 9.50±0.5).Therefore in the case of plasma and heart ,Group III &IV are more effective (P<0.05) in decreasing MDA compared to Group I control animals, but this significant is not observed in aorta .In plasma, rats supplemented with the diet containing 5% dried mushroom Gr.IV had higher (P<0.05) MDA compared to these supplemented with MR alone control group except heart.

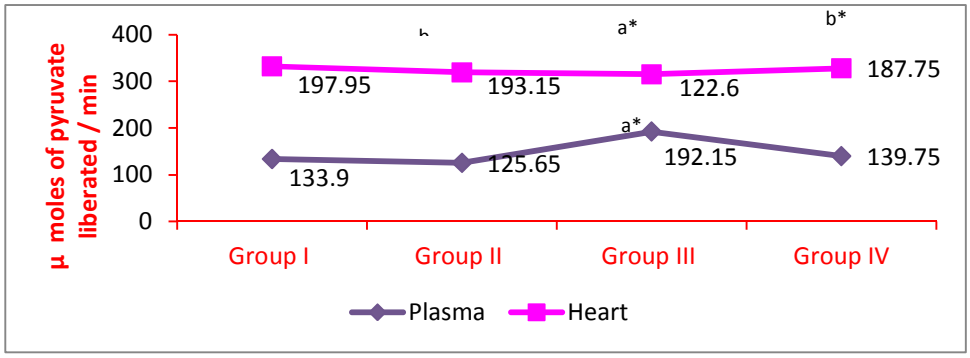
Fig 2.Level of creatine kinase in control and experimental animals



Values are expressed as mean ± SD for six animals in each group
Comparisons were made as in Fig 1. Statistically significant alterations are expressed as *P<0.05.

Fig (2) shows administration of 5% mushroom treated HFD Group IV animals for 105 days increase heart CK level from (10.88±0.60 to 19.25±0.92).An increase in CK level in Group IV animals are more effective(P<0.05) compared to Group III ,HFD fed animals. Group III ,HFD diet animals show a reduction in CK level ,the decrease being greater in MR treated groups and also more significant (P<0.05) compared to Group I, control animals. In plasma, the atherogenic diet induced abnormal rise in the activities of creatine kinase, concentrations is restored to near control values in the treated group. Therefore, after five weeks of therapy, the creatine kinase had significantly declined from 787.10±9.48 to 519.15±12.52 in plasma while significant change in the levels of the heart creatine kinase (p>0.05) was observed.

Fig 3.Level of Lactate dehydrogenase in control and experimental rats



Values are expressed as mean ± SD for six animals in each group.
Comparisons were made as in Fig 1. Statistically significant alterations are expressed as *P<0.05.

Fig (3) presents the results of plasma and heart LDH activities in the control and experimental groups. There was significant increases (p<0.05) in the plasma LDH activities of hypercholesterolemic rats (HC) as compared to normal control rats(NC).Whereas there was significant decrease in the heart LDH activities of HF induced rats as compared to normal control rats. In plasma and heart there were no changes in LDH activity observed by group II animals when compared with hypercholesterolemic and 5% mushroom treated groups. The plasma dehydrogenase (LDH) enzyme in rats was assessed to significantly (P<0.05) reduced by the supplementation of high fat diet with 5%mushroom reduced LDH enzyme activity by 192.15±3.04 to 139.75±3.75 in plasma, whereas in the heart lactate dehydrogenase enzyme in rats was assessed to significantly(P<0.05) increased by the supplementation of high fat diet with 5% mushroom

increased LDH enzyme activity by from 122.60 ± 8.20 to 187.75 ± 2.05.Wistar albino rats supplemented with 5% mushroom showed a higher (P<0.05) LDH enzyme compared to those supplemented with mushroom. Diet supplemented with 5%mushroom in high fat diet of Group IV is significant when compared to Group II animals, but it is not significantly (P>0.05)differed in their impact on LDH enzyme of heart of Group II fed animals. Further, diet supplemented with mushroom had a lower lactate dehydrogenase (LDH) enzyme compared to that of the Group IV of plasma and had a higher lactate dehydrogenase (LDH) enzyme compared to that of Group IV fed animals of heart. Mushroom reduced LDH enzyme by 139.75±3.75 in plasma and higher 187.75±2.05 in heart respective. However, no studies have focused on the effect of mushroom, Agaricusbisporus on LDH and hypercholesterolemia.

3.1. Discussion

In biological environments, the most favourable substrate for lipid peroxidation is represented by polyunsaturated fatty acids. Hypercholesterolemia mediated atherosclerosis is associated with an increase in the level of the lipid peroxidation product, malondialdehyde (MDA), which is an index of the level of oxygen free radicals[24]; it also reacts with polyunsaturated fattyacids, causing free radical mediated tissue damage in cellular membranes. The poly unsaturated fatty acids in the cell membrane are protected against lipid peroxidation through endogenous antioxidants such as α-tocopherol [25]. A decrease in lipid peroxidation leads to reduction in arterial wall cholesterol content. Therefore , reduction of atherosclerosis caused by hypercholesterolemia is associated with a decrease in lipid peroxidation, while increased lipid peroxidation is a characteristic feature of hypercholesterolemia; it impairs cell membrane fluidity and alters the activity of membrane bound enzymes and receptors, resulting in membrane malfunction[26].

Mushroom might be effective in prevailing lipid peroxidation produced by increased levels of lipids induced by HFD. In the present study ,the table 1 shows that the plasma and heart MDA have been increased on atherogenic diet feeding (Group III).The oral administration of mushroom to hypercholesterolemic rats resulted in significantly lower mean levels of MDA in plasma and heart. The decrease in intensity of lipid peroxidation , as inferred from the lower mean levels of MDA ,was possibly due to the free radical scavenging property of the hydroxyl groups.

In the present study ,the mean activities of plasma and heart LDH were significantly higher in hypercholesterolemic, HFD treated rats than those in control rats (Fig 1).

However, such elevations in the mean levels of serum and heart LDH enzyme appear to have been prevented in hypercholesterolemic rats that had been treated with the MR, since the mean levels were significantly lower than hypercholesterolemic, mushroom treated rats. These observations suggest that the piper betle extract and eugenol were able to the hepatic tissue from hypercholesterolemia induced oxidative stress mediated cellular damage .These results are consistent with those of an earlier study, in which the mean serum levels of LDH was found to be significantly lower in rats with Triton WR-1339 induced acute hypercholesterolemia that had been treated with a mushroom extract or with chrysin[27].

Filipek *et al* .,[28] reported that mushrooms must have antioxidative ability which might be caused by a relatively high content of chitin because some carboxy methy lglucanes were found to have the ability to quench hydroxyl radicals. It would be that the antioxidative ability of Pleurotusostreatus plays in important role because a relationship between the breakdown of lipid metabolism and lipid peroxidation is confirmed. These factors would apply to the actual results for the experiments carried out to the mushrooms.

In accordance with Sharma *et al*., [29], lipid peroxidation results in the formation of MDA and therefore, the rise in the MDA content of blood platelets and aortic tissue would suggest involvement of OFRs in the tissue damage. The severity of aortic atherosclerotic lesions in animals fed with the high cholesterol diet was significantly reduced on supplementation with vitamin E. The protective effect of vitamin E could possibly be attributed to the decreased content of MDA in the aortic tissue [30]. The vitamin E effectively serves as the major lipid-soluble chain-breaking antioxidant, preventing lipid peroxidation and modulating the metabolism of the arachidonic acid cascade initiated by lipooxygenase and cyclooxygenase despite its low molar conentrations in the membrane [31].It has earlier been reported that administration of L-carnitine to rats intoxicated with ethanol significantly protects lipids and proteins against oxidative modifications in the serum and liver. The level of MDA was decreased by about 30%in the blood serum in comparison to the ethanol group[32].

The effect on serum creatine kinase of simvastatin, a recently available antilipemic drug,as a monotherapy for hyper holesterolemic patients. The subjects consuming the allicin (garlic) reported a reduced rate of perceived exertion, and had significantly lower plasma levels of CK, CK-MM (muscle-specific) when compared with the control group[33]. However, there are many patients in statins who have no symptoms, but have high CK in their blood, with excellent LDL lowering, and without muscle pain or damage [34].

There was significant decrease in the plasma LDH activity of chitosan in hypercholesterolemic rats. In hypercholesterolemia the increase in the plasma activities of LDH was directly proportional to the degree of cellular damage. These values decreased by chitosan [35].

4. Conclusion

Conclusively, mushroom is an effective oral antiperoxidative agent for treating HF diet induced hypercholesterolemic rats. Therefore dried mushroom can improve the antiperoxidative status, and minimize the occurrence of cellular damage. Dietary restriction induced a significant reduction in the CK and LDH enzyme level.

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