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Antioxidant Activity of *Agaricusbisporus* in Hypercholesterolemic Rats.

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Abstract: The objective of the study is to investigate the effect of MR, *Agaricusbisporus* on the activities of ascorbic acid, α -tocopherol, uric acid, glutathione, CAT, SOD, GPX and GST in diet induced atherosclerotic rats. *Agaricusbisporus* was selected for the reasons that it contains nutrients such as proteins, minerals etc., in higher quantities than other species due to which it has high antioxidant property. The animals were divided into four groups consisting of six rats per group. Group I-control animals; The Group II- MR treated rats; the Group III- high fat diet (HFD) fed animals; and the Group IV-HFD and 5% dry mushroom powder fed animals. Rats were fed for 105 days and then blood collection from the sinus venosus, plasma was separated. Aorta and heart were dissected out and washed and homogenized. The assays were carried out on the sameday. While putting all the facts of study in a nutshell, it is very clearly pointed out that the level of non enzymic antioxidants like ascorbic acid and α -tocopherol in plasma and glutathione in aorta and heart demonstrated a sharp decrease in experimental rats. Whereas mushroom treatment moderately elevate the antioxidant levels. But in the case of uric acid, the HFD fed rats the level in RBC membrane was elevated. Administration of MR marginally decreased in the level of antioxidant. The activities of antioxidant enzymes such as CAT, SOD, GPX and GST in aorta and heart were declined significantly in experimental animals. Treatments with MR markedly improved the overall antioxidant status of the atherosclerotic animals. Many indigenous plants have gained importance in the prevention and treatment of coronary heart disease, but they demand systematic and scientific evaluation. Recent reports have shown that mushroom are good for diet and have a significant therapeutic effect. This provoked us to investigate the role of edible mushrooms *Agaricusbisporus* in hypercholesterolemia.

Keywords: *Agaricusbisporus*, catalase, edible mushroom, glutathione peroxidase, glutathione-s-transferase, superoxide dis mutase and uric acid.

Abbreviations: Ag: *Agaricusbisporus*; MR: Mushroom; HFD: Highfatdiet; CAT: Catalase; SOD: Superoxide dismutase; GPX: Glutathioneperoxidase; GST: Glutathione-S-transferase; Vit C: Ascorbic acid.

1. Introduction:

The objective of the study is to investigate the effect of MR supplementation on the activities of plasma ascorbic acid, α -tocopherol, erythrocyte membrane uric acid, level of glutathione in plasma, aorta and heart, the level of antioxidant enzymes such as CAT, SOD, GPX and GST in aorta and heart in hypercholesterolemic rats.

Hypercholesterolemia is one of the major complications in health of the people all over the world, which mainly contribute to cardiac diseases and hypertension. Hypercholesterolemia is also related to diabetes and it has role in inducing oxidative stress [1]. Since the ancient times, mushrooms have been regarded as important

medicinal food items, which have preventing and protective effects against many disorders, including hypercholesterolemia.[2].

Living cells, including those of man, animals and plants are continuously exposed to a variety of challenges that exert oxidative stress. Oxidative stress arises in a biological system after an increased exposure to oxidants, a decrease in the antioxidant capacity of the system, or both. It is often associated with or leads to the generation of reactive oxygen species (ROS), including free radicals, which are strongly implicated in the pathophysiology of diseases, such as cancer, rheumatoid arthritis, cirrhosis and arteriosclerosis as well as in degenerative processes associated

with ageing. Reactive free radicals may come from endogenous sources through normal physiological and metabolic processes such as mitochondrial respiration. Alternatively, they could result from exogenous sources such as exposure to pollutants and ionizing irradiation and particularly oxygen derived radicals are capable of oxidizing biomolecules, resulting in cell death and tissue damage^[3].

Cells are equipped with several defence systems against free radical damage, including oxidative enzymes such as superoxide dismutase (SOD) and catalase (CAT), or chemical compounds such as α -tocopherol, ascorbic acid, carotenoids, polyphenol compounds and glutathione^[4]. However, antioxidant supplements or antioxidant containing foods may be used to help the human body to reduce oxidative damage or to protect food quality by preventing oxidative deterioration^[5]. The antioxidants contained in foods, especially vegetables are phenolic compounds, carotenoids, tocopherol and ascorbic acid that are important protective agents for human health^[6].

Mushrooms, popular in Chinese medicine and food fields, are highly medicinal and nutritious. Recently, many compounds with antitumor and antioxidant potential have been isolated and identified from medicine, edible and wild mushrooms^[7]. Moreover, these mushrooms have currently received great attention due to the therapeutic effects such as antitumor, immune modulating and antioxidant activities^[8] more evidences have been reported that many potential antioxidants can be isolated from mushroom sources. Antioxidant activity is one of the most important bioactivities of polysaccharides from various mushrooms. It was reported that antioxidant properties of mushroom polysaccharides are related to their molecular weight and protein/polysaccharide ratios. Several researchers have found that many types of polysaccharides produced by submerged cultures of mushrooms process effective antioxidant properties^[9].

Many species of mushrooms like *Ganoderma lucidum*, *Lentinus edodes*, *Grifola frondosa*, *Cordyceps sinensis* and even some *Pleurotus* spp like *P. ostreatus* have scientifically proved antioxidant activities^[10]. This property of mushroom is supposed to be effective also against the hypercholesterolemia induced oxidative alterations.

This work was designed to evaluate the antioxidant power of one of the most common cultivated type of mushrooms in South India on

the oxidative stress generated in hypercholesterolemic rat model.

2. Materials and Methods

2.1. Chemicals

Cholesterol, cholin chloride, sodium cholate, 2-thiouracil, α -tocopherol, reduced glutathione were purchased from Sigma chemicals (St. Louis, USA). 2,4-dinitrophenyl hydrazine, dithionitrobenzoic acid, 1-chloro, 2,4-dinitro benzene, ascorbic acid, dipyrindyl, sodium azide and all the other chemicals and reagents used were of analytical grade and were obtained from SD fine chemicals, Mumbai, India.

2.2. Housing and Management of Animals

Male albino rats of Wistar strain with an initial body weight of about 265-275 gm were used for this study^[11]. 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".

2.3. Experimental Protocols

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 hypercholesterolemia 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.,^[12] 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^[13]. 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 thio uracil. 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.

2.4. 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 . 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^[14].

2.5. 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°C) and assays were made on the same day.

The level of plasma ascorbic acid was estimated by the method of Omaye *et al.*,^[15]. The level of plasma vitamin E was determined by the method of Quaipe *et al.*,^[16] with little modification by Baker and Frank,^[17]. The level of erythrocyte membrane uric acid was determined by the method of Dodge *et al.*,^[18] with a change in buffer according to Quist,^[19]. The level of plasma and tissue reduced glutathione (GSH) was determined by the method of Moron *et al.*,^[20]. Tissue catalase activity was assayed by the method of Sinha,^[21]. Tissue superoxide dismutase(SOD) activity was assayed in the homogenate according to the method of Misra and Fridorich, 2, based on the oxidation of epinephrine adrenochrome transition by the enzyme. Tissue activity of glutathione peroxidase (GPX) was determined by the method of Rotruck *et al.*,^[22] with some modifications. Tissue glutathione-S-transferase was assayed by the method of Habig *et al.*,^[23].

2.6. Statistical Methods

The data obtained were analyzed using the one way analysis of variance[ANOVA] to determine differences^[24] and Tukey's Multiple Range Test[TMRT] to separate the means \pm SD. The P value <0.05 was considered to be statistically significant.

3. Result

It is clear from Table I that non-enzymic antioxidant in control and experimental albinorats with reference to plasma ascorbic acid, α -tocopherol are very significant except uric acid .The explanation of significance is clearly pointed out below:

Table 1: Level of plasma and erythrocyte membrane non-enzymic antioxidants in control and experimental rats.

Investigation	Group I C	Group II C+MR	Group III HFD	Group IV HFD + MR
Plasma				
Ascorbic Acid	4.10 \pm 0.14 ^a	3.58 \pm 0.03	2.15 \pm 0.21 ^{a*c}	5.20 \pm 0.14 ^{a*c*}
α -tocopherol	20.65 \pm 1.34 ^a	19.20 \pm 0.28	17.45 \pm 2.19 ^{a*c}	21.38 \pm 1.48 ^{c*}
Erythrocyte Membrane				
Uric acid	3.95 \pm 0.07	3.70 \pm 0.28	6.40 \pm 0.42 ^c	3.20 \pm 0.28 ^{c*}

Values are expressed as mean \pm SD for six animals in each group. Ascorbic acid and α -tocopherol μ g/mg protein; Uric acid mg/dl. Comparisons were made as follows:

- i. Group III, IV are compared with Group II denoted as "a".
- ii. Group IV is compared with Group II, denoted as "b".
- iii. Group IV is compared with Group III denoted as "c".

Statistically significant alterations are expressed as * p < 0.05.

Administration of HFD for fifteen weeks, decreases plasma ascorbic acid and α -tocopherol from 4.10 μ g to 2.15 μ g/mg protein, 20.65 μ g to 17.45 μ g/mg protein (Gr III) and increases erythrocyte membrane uric acid from 3.95 mg to 6.40 mg/dl (Gr III). Group IV animals shows a increases in plasma ascorbic acid and α -tocopherol except erythrocyte membrane uric acid.

The increase in plasma ascorbic acid and α -tocopherol level observed in MR treated groups (Gr IV) whereas decrease in erythrocyte membrane uric acid level observed in MR treated

groups (Gr IV). Treatment of normal animals with 5% MR treated animals slightly altered plasma ascorbic acid and α -tocopherol and also erythrocyte membrane uric acid. Atherosclerotic rats show decreased level of plasma ascorbic acid and α -tocopherol but uric acid is increased in erythrocyte membrane. The concentration of ascorbic acid, α -tocopherol and uric acid were influenced by the dietary cholesterol level whereas that of all non-enzymic antioxidant level tended to increase gradually in response to the 5% dietary mushroom level (GrIV) except uric acid.

Table 2. Level of glutathione in plasma, aorta and heart of control and experimental rats.

Investigation Glutathione	Group I C	Group II C+MR	Group III HFD	Group IV HFD + MR
Plasma	1.74 \pm 0.04	1.07 \pm 0.08 ^b	1.99 \pm 0.01	1.62 \pm 0.02 ^{b*}
Aorta	8.70 \pm 0.14 ^a	8.20 \pm 0.28	4.32 \pm 0.45 ^{a*c}	8.20 \pm 0.28 ^{c*}
Heart	9.00 \pm 0.14 ^a	7.65 \pm 0.21 ^b	4.30 \pm 0.14 ^{a*c}	9.25 \pm 0.21 ^{b*c*}

Values are expressed as mean \pm SD for six animals in each group.
Glutathione - μ g/mg protein.

Table(2) depicts the level of reduced glutathione in the plasma, aorta and heart of normal and experimental groups of rats. There was decreases in the plasma, aorta, heart reduced glutathione activities of hypercholesterolemic rats (HC) as compared to normal control rats (NC). In plasma and heart there were significant in glutathione activity observed by group II animals when compared with hypercholesterolemic and 5% mushroom treated groups except aorta glutathione in 5% mushroom treated groups. The non-enzymic antioxidant activity in rats were assessed to significantly ($p < 0.05$) increased by the

supplementation of high fat diet with 5% mushroom increased plasma glutathione activity by 1.62 \pm 0.01 to 1.99 \pm 0.02, aorta glutathione activity by 4.32 \pm 0.45 to 8.20 \pm 0.28 and heart glutathione activity by 4.30 \pm 0.14 to 9.25 \pm 0.21.

The administration of *Agaricus bisporus* significantly increased the HFD induced adverse effects and maintained the level of evaluated parameters at near normalcy. In group II rats the oral administration of *Agaricus* resulted in a significant ($p < 0.05$) elevated in the level of reduced glutathione.

Table 3. Level of antioxidant enzymes in aorta of control and experimental rats

Investigation	Group I C	Group II C+MR	Group III HFD	Group IV HFD + MR
CAT	6.50 \pm 0.14 ^a	6.25 \pm 0.21	5.25 \pm 0.07 ^{a*c}	10.88 \pm 0.11 ^{c*}
SOD	4.83 \pm 0.18 ^a	4.78 \pm 0.25	3.38 \pm 0.11 ^{a*c}	7.50 \pm 0.14 ^{c*}
GPX	4.72 \pm 0.32 ^a	4.53 \pm 0.25	3.15 \pm 0.21 ^{a*c*}	7.10 \pm 0.14 ^{c*}
GST	1333.25 \pm 4.60 ^a	1370.35 \pm 4.03 ^b	1137.95 \pm 1.91 ^{a*c}	1267.75 \pm 3.89 ^{b*c*}

Values are expressed as mean \pm SD for six animals in each group.

Activity of enzymes are expressed as follows:

Catalase: units of H_2O_2 utilised/min/mg of protein;

SOD : units (1 unit =the amount of enzyme required to bring about 50% inhibition in the autoxidation)/min/mg of protein;

Gpx: μ g of GSH utilised /min/mg of protein;

GST: nmoles of CDNB conjugated in min/mg of protein .

Table (3) presents the results of aorta CAT, SOD, GPX and GST activities in the control and experimental groups. There was decreases in the aorta CAT, SOD, GPX and GST activities of hypocholesterolemic rats(HC) as

compared to normal control rats (NC). In aorta there were no significant in CAT, SOD, GPX and GST activity observed by group II animals when compared with hypercholesterolemic and 5% mushroom treated groups except GST in 5%

mushroom treated groups. The aorta antioxidant enzymes in rats were assessed to significantly ($p < 0.05$) increased by the supplementation of high fat diet with 5% mushroom increased CAT enzyme activity by 5.25 ± 0.07 to 10.88 ± 0.11 . SOD enzyme activity by 3.38 ± 0.11 to 7.50 ± 0.14 , GPX enzyme activity by 3.15 ± 0.21 to 7.10 ± 0.14 and GST enzyme activity by 1137.95 ± 1.91 to 1267.75 ± 3.89 in aorta. In GST, 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 CAT, SOD, GPX enzymes of aorta of Group II fed animals. Further, diet supplemented with mushroom had a lower CAT, SOD, GPX enzyme compared to that of the Group IV of aorta and had a higher GST enzyme compared to that of Group IV fed animals of aorta. Mushroom increased CAT, SOD, GPX and GST enzymes by 10.88 ± 0.11 , 7.50 ± 0.14 , 7.10 ± 0.14 and 1267.75 ± 3.89 in aorta respectively.

Table 4: Level of antioxidant enzymes in heart of control and experimental rats.

Investigation	Group I C	Group II C+MR	Group III HFD	Group IV HFD + MR
CAT	8.48 ± 0.19^a	8.45 ± 0.49	$3.98 \pm 0.02^{a*c}$	$7.85 \pm 0.07^*$
SOD	5.69 ± 0.12^a	5.60 ± 0.07^b	$2.83 \pm 0.11^{a*c}$	$4.77 \pm 0.25^{a*b*c*}$
GPX	7.65 ± 0.21^a	7.60 ± 0.42^b	$2.63 \pm 0.17^{a*c}$	$6.15 \pm 0.14^{a*b*c*}$
GST	1690.25 ± 2.47^a	1660.70 ± 1.84^b	$1312.30 \pm 15.98^{a*c}$	$1623.30 \pm 4.10^{a*b*c*}$

Values are expressed as mean \pm SD for six animals in each group. Activity of enzymes are expressed in Table 3.

Table 4 presents the results of heart CAT, SOD, GPX and GST activities in the control and experimental groups. There were significant decreases in the heart CAT, SOD, GPX, GST activities of HF induced rats as compared to normal control rats. In heart there were no significant in CAT, SOD, GPX and GST activity observed by group II animals when compared with hypercholesterolemic groups, and also there were significant in SOD, GPX and GST activity observed by group II animals when compared with 5% mushroom treated groups. The heart antioxidant enzymes in rats were assessed to significantly ($p < 0.05$) increased by the supplementation of high fat diet with 5% mushroom increased CAT enzyme activity by 3.98 ± 0.02 to 7.85 ± 0.07 , SOD enzyme activity by 2.83 ± 0.11 to 4.77 ± 0.25 , GPX enzyme activity by 2.63 ± 0.17 to 6.15 ± 0.14 and GST enzyme activity by 1312.30 ± 15.98 to 1623.30 ± 4.10 in heart. In SOD, GPX and GST 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 CAT enzyme of heart of Group II fed animals. Further, diet supplemented with mushroom had a lower GST enzyme compared to that of Group IV of heart and had a higher CAT, SOD, GPX enzymes compared to that of Group IV fed animals of heart. Mushroom increased CAT, SOD, GPX and GST enzymes by 7.85 ± 0.07 , 4.77 ± 0.25 , 6.15 ± 0.14 and 1623.30 ± 4.10 in heart respectively.

3.1. Discussion

Recent studies suggest that increased free radical formation and subsequent oxidative stress associated with occurrence of a relative deficit in the endogenous antioxidants, may be one of the mechanism for the development of heart failure after myocardial infarction^[25].

The present investigation is designed to analyse the quantity of plasma vitamin C in Group III (HFD animals), Group IV (HFD+MR) animals. The results obtained indicate that there was steady increase in Group IV (5.20 ± 0.14). Similar observation was seen by Jeserich *et al.*,^[26] in patients with hypercholesterolemia. Therefore, it is quite evident that formation of free radicals increases in hypercholesterolemia or hypertension due to inactivation of the vasodilator.

According to Godfried and Combs^[27], the levels of vitamin E in the aortic tissue have not been measured but a dose dependent accumulation of α -tocopherol in arterial walls and liver of rabbit has been reported.

Plotnick *et al.*,^[28] pointed out that an oral dose of 1000mg of vitamin C in combination with 800 IU of vitamin E exhibited normal vasodilation several hours after a single high fat meal, whereas control subjects which were not given the antioxidant combination showed impaired vaso reactivity. The effect of MR showed increased activities of antioxidant enzymes.

Uric acid is the final break down product of purine degradation in humans. Although

elevated uric acid is most intimately associated with gout.

Gertler and Coworkers,^[29] observed nearly fifty years ago, an association between serum uric acid and coronary heart disease. Serum uric acid levels were directly associated with most CHD risk factors including hypertension, systolic blood pressure, LDL cholesterol, triglycerides and low HDL cholesterol.

Freedman *et al.*,^[30] hence suggested serum uric acid might be a stronger predictor of CHD in women than men. These levels would become elevated from either increased urate production or from decreased uric acid excretion. The results show that increased uric acid levels at Group III, HF diet, mushroom supplementation at Group IV (HFD+MR) leads to decreased levels of RBC membrane uric acid (Group IV). Elevated serum uric acid could be a compensatory mechanism to counteract oxidative damage related to atherosclerosis and aging in humans^[31].

GSH status is highly sensitive indicator of cell functionality and viability. It is a ubiquitous thiol containing tripeptide, which plays a central role in cell biology. It is implicated in the cellular defences against xenobiotics and naturally occurring deleterious compounds such as free radicals and hydroperoxides. GSH depletion is linked to a number of disease states including cancer, neurodegenerative and cardiovascular diseases. Glutathione not only protects cell membranes from oxidative damage, but also helps to maintain the sulphhydryl groups of many proteins in the reduced form, requirements for their normal function^[32].

In the present study, the reduction noticed in the level of GSH in plasma, aorta, heart of HFD induced atherosclerotic was either due to increased degradation or decreased synthesis of glutathione. Depletion of GSH results in HFD+MR (Gr.IV) consumption as observed in the present study. Pretreatment with *Agaricus bisporus* prevented the HFD induced atherosclerosis and maintained the level of reduced glutathione near normal level in plasma, aorta and heart. This is due to antioxidant activity of *Agaricus bisporus*.

Frei,^[33] stated that intracellular glutathione plays a central role in protecting endothelial cells against oxidized LDL and other oxidant insults such as hydrogen peroxide. In addition, cellular antioxidants also play an important role in maintaining the normal production and biological activity of EDNO, nitric oxide is of central importance in both

atherosclerosis and its clinical manifestations (i.e., angina pectoris, myocardial infarction and stroke) as it inhibits endothelial leukocyte interactions, smooth muscle cell proliferation and platelet aggregation and causes vasodilation. Glutathione is present intracellularly in millimolar concentrations, inhibits oxidation of tetrahydrobiopterin, which is a cofactor for the nitric oxide synthase reaction, or stimulate endothelial L-arginine uptake, which is a substrate for nitric oxide synthase^[34].

In this study, it has been found that all the studied parameters such as glutathione, ascorbic acid, α -tocopherol, CAT, SOD, GPX and GST were altered in the plasma, aorta and heart of atherosclerotic rats significantly except uric acid activity was higher in HFD, Gr.III, than in HFD+MR, Gr.IV, but the difference was significant. Feeding of *Agaricus bisporus* recovered the alterations in some degrees. And most importantly, *Agaricus bisporus* fed groups (HFD+MR respectively) had significantly higher glutathione, ascorbic acid, α -tocopherol activity in plasma, higher glutathione, CAT, SOD, GPX and GST activity in aorta and heart except uric acid in erythrocyte membrane.

These findings suggest that these mushrooms are potential to recover and improve the alteration in antioxidant systems by atherosclerosis. The antioxidant activities of these mushrooms increasing glutathione, ascorbic acid, α -tocopherol, CAT, SOD, GPX and GST activities may be due to their nutritional or chemical composition. Vitamin E is regarded as an important natural antioxidant. *Agaricus* mushroom contains a considerable amount of vitamin E^[35].

As Bobek,^[36] has been reported that the addition of tomato and grape pomace to the cholesterol (0.3%) diet of male Wistar rats produced a dose dependent effect. Diets containing tomato and grape pomace showed a tendency towards higher SOD and GPX activity in the liver.

Godfried *et al.*,^[37] had proved that the increase in the levels of OFRs was existed in hypercholesterolemia due to the decrease in the activity of OFR metabolizing enzymes like CAT, SOD, GPX. These antioxidant enzymes normally protected the tissues from oxidant injury. The results show that the levels of antioxidant enzymes are increased in the group of animals receiving MR (Group IV).

According to Clare *et al.*,^[38] catalase activity was noted higher in rats fed fish oils, suggesting that feeding fish oils to rats in early

life raises oxidative stress throughout life. The antioxidant activities of *P.ostreatus* and other mushrooms have been reported earlier^[39]. Also a few reports exist about the antioxidant and antitumor role of *P.florida*^[40].

Therefore, this study was performed to monitor the change of antioxidant activities in therapeutic potential of mushroom and its antioxidant effect in atherosclerotic induced albino rats. As, atherosclerotic induces cellular oxidative stress, to protect cells or tissues antioxidant systems have to fight against this. Due to hypercholesterolemia, the antioxidant systems are supposed to be altered usually.^[41]

So, this mushroom is recommended for the common people as a nutritious and health beneficial food supplement. The findings of this study suggest that in addition to nutritional and antiatherosclerotic importance, *Agaricusbisporus* have roles in recovering and improvement of antioxidant systems of atherosclerotic rat plasma, erythrocyte membrane, aorta and heart atleast in some degress.

4. Conclusion

While putting all the facts of study in a nutshell is very clear that the level of non-enzymic antioxidants like plasma ascorbic acid, α -tocopherol demonstrated a sharp decrease in experimental rats, whereas erythrocyte membrane uric acid demonstrate a sharp increase in experimentalrats. As regards , antioxidant enzymes like CAT, SOD,GPX and GST in aorta and heart were declined significantly in experimental animals. Treatment with MR markedly improved the overall antioxidant status of the atherosclerotic animals. This area of work which dealt with the dietary supplementation with mushroom is to hither unexplored and needs immediate attention and the meager available evidence in literature in this aspect indicates a bright future for coronary heart disease victims.

If mushroom therapy proves useful in rodents which will be elucidated in this study this observation could be extrapolated to human cases and if this effort is achieved hundreds of lives could be saved ever year .

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