

## Beneficial Role of Mushroom in Recovering Complications of Hypercholesterolemia

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**Abstract.** Mushrooms are considered as a valuable source of important nutrients having hepatoprotective and anti-hyperlipidemic actions. Present experimental research was done to explore the beneficial role of mushroom on health in hypercholesterolemia. Total thirty Swiss albino mice were taken and randomly divided into three groups: control A, group B and group C. Each group consisted of ten mice. The control A group was fed with normal mice pellet and fresh water. Group B was fed with hypercholesterolemic diet and group C was supplied hypercholesterolemic diet with mushroom powder (500g/kg/mice body weight) for 60 days. After the experimental tenure, mice of each group were sacrificed ethically and the samples (liver and blood) were collected for gross, histological study and lipid profile analysis. Increased liver weight, pale and hemorrhagic liver in gross observation along with some histological changes including dilation and congestion of central and portal vein, fat accumulation in hepatocyte and marked lymphocytic infiltration were found in group B, while mushroom supplementation recovered this gross and histological changes and reduced liver weight in group C. Just mild congestion and dilation was in the portal vein of group C. In lipid profile analysis, total cholesterol (TC), triglyceride (TG) and low density lipoprotein (LDL) level significantly reduced respectively by 10%, 38% and 17% in group C than group B. High density lipoprotein (HDL) level also significantly increased by 20% in group C compared to group B. Therefore, it can be concluded that mushrooms might have potentially beneficial actions in recovering of some complications in hypercholesterolemia.

**Keyword:** Hepatoprotective, Hypocholesterolemic properties, Hypercholesterolemia, Mushroom, Swiss albino mice.

**Abstrak.** Jamur dianggap sebagai sumber nutrisi penting yang berharga yang memiliki aktivitas sebagai hepatoprotektif dan anti hiperlipidemia. Penelitian eksperimental ini dilakukan untuk mengeksplorasi peran menguntungkan jamur pada kesehatan salah satunya hiperkolesterolemia. Mencit Swiss albino sebanyak tiga puluh ekor diambil dan dibagi secara acak menjadi tiga kelompok yaitu kontrol A, kelompok B dan kelompok C. Setiap kelompok terdiri dari sepuluh ekor mencit. Kelompok kontrol A diberi pakan pelet tikus normal dan air tawar. Kelompok B diberi diet hiperkolesterolemia dan kelompok C diberi diet hiperkolesterolemia bubuk jamur (500g/kg/berat badan tikus) selama 60 hari.

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Setelah masa percobaan, tikus dari masing-masing kelompok dikorbankan secara etis dan sampel (hati dan darah) dikumpulkan untuk studi makroskopis, histologis dan analisis profil lipid. Peningkatan berat hati, hati pucat dan hemoragik dalam pengamatan bersama dengan beberapa perubahan histologis termasuk pelebaran dan kongesti vena sentral dan portal, akumulasi lemak di hepatosit dan infiltrasi limfositik yang nyata ditemukan pada kelompok B, sementara suplementasi jamur memulihkan perubahan besar dan histologis ini dan penurunan berat hati pada kelompok C. Hanya kongesti dan pelebaran ringan pada vena portal kelompok C. Dalam analisis profil lipid, kadar kolesterol total (TC), trigliserida (TG) dan lipoprotein densitas rendah (LDL) berkurang secara signifikan masing-masing sebesar 10%, 38% dan 17% pada kelompok C dibandingkan kelompok B. Tingkat high density lipoprotein (HDL) juga meningkat secara signifikan sebesar 20% pada kelompok C dibandingkan dengan kelompok B. Oleh karena itu, dapat disimpulkan bahwa jamur mungkin memiliki tindakan yang berpotensi bermanfaat dalam pemulihan beberapa komplikasi pada hiperkolesterolemia.

**Kata Kunci:** Hepatoprotektif, Hiperkolesterolemia, Jamur, mencit Swiss albino.

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## 1 Introduction

Mushrooms are considered as an important food items for their nutritional value and therapeutic properties [1]. Since ancient times, mushrooms have been consumed by human as normal diet and also as delicious foods due to its highly desirable taste and aroma [2]. Mushrooms contain high moisture percentage based on harvest, growth and storage conditions [3]. *Agaricus bisporus* (white button mushroom; WBM) contains high level of dietary fibers and antioxidants including vitamin C, D, and B12, folates and polyphenols. Dry *Agaricus bisporus* fruit bodies contains carbohydrate 48.9-38.3%, fibers 23.3-17.7%, ash 11.00-7.77%, and fat 3.92-2.53% [4]. It is also a good source of B vitamins such as riboflavin, niacin and pantothenic acid, selenium copper, phosphorus, zinc, potassium, minimal or no vitamin C and sodium [5]. Nutritional components (% in grams) of edible mushroom (*Agaricus bisporus*) [6] have also been enlisted in (Table 1). Cardiovascular diseases are associated with daily diet [5]. Diet with high saturated fatty acids increase LDL cholesterol level and cause cardiovascular diseases [7]. Most research stated that medical mushrooms had beneficial role on blood cholesterol and cardiovascular diseases [8]. In clinical practices, bioactive compounds derived from the extract of mushroom have been widely used for the prevention and treatment of diseases such as diabetes, cancer, immune system disorders and infections [9]. The main beneficial role of mushroom includes cholesterol and blood pressure lowering properties, liver protective, antidiabetic and antimicrobial activities [10,11].

**Table 1.** Chemical constituents of fresh and conserved sample of mushroom (*Agaricus bisporus*) (% in grams)

Chemical constituents	<i>Agaricus bisporus</i> fresh	<i>Agaricus bisporus</i> conserved
DM (%)	9.62±1.04	8.23±0.73
Crude protein (DM %)	34.84±0.05	40.6±0.4

Crude fat (DM %)	2.28±0.02	2.30±0.015
Crude ash (DM %)	9.23±0.06	8.34±0.12

Mushrooms accumulate a variety of secondary metabolites, including phenolic compounds, terpenes and steroids where a phenolic compound has been found to be an excellent antioxidant and synergist that is not mutagenic. Antioxidant compounds prevent oxidative damage related to aging and diseases, such as atherosclerosis, diabetes, cancer, and cirrhosis. Mushrooms that contain antioxidants or increase antioxidant enzyme activity may be used to reduce oxidative damage. These appear to be the main endogenous sources of most of the oxidants produced by cells. Exogenous sources of free radicals include tobacco smoke, ionizing radiation, certain pollutants, organic solvents and pesticides. They are capable of attacking the healthy cells of the body, causing them to lose their normal structure and function [12].

Medicinal mushroom is an excellent natural sources of therapeutic agent and also scientifically known as efficacious and safe medicinal herbs [13]. *Agaricus bisporus* is low in fat content but they contain some essential fatty acids such as linoleic acid. *Agaricus bisporus* contains 20- and 5-folds more linoleic acid than *Ganoderma lucidum* and *Pleurotus ostreatus* respectively [14]. This linoleic acid is essential for human health and reduces atherosclerosis [15]. Triterpenoids extract of *Ganoderma lucidum* (75% ethanol) can protect mice against hepatic necrosis induced by chloroform and d-galactosamine [16]. Polysaccharides derived from mushrooms exhibit diverse activities that have been isolated from *Agaricus bisporus* and *Coprinus comatus* [17,18]. Mushrooms in daily diet could significantly decrease ( $*p < 0.05$ ) total cholesterol (TC), triglyceride (TG), low-density lipoprotein (LDL) and significantly increase ( $*p < 0.05$ ) high density lipoprotein (HDL) level [19]. Mushrooms intake creates an effective influence on some metabolic markers (total cholesterol, LDL, HDL cholesterol, triglyceride, blood pressure, homeostatic function and oxidative inflammatory damage) that potentially reduce the risk of heart diseases. Edible mushrooms reduce cardiovascular risk factors such as low-density lipoprotein (LDL) cholesterol and high total cholesterol (TC), atherosclerosis, high blood pressure and oxidative and inflammatory damage [20]. *Agaricus bisporus* supplementation significantly corrected diet induced hypercholesterolemia [21]. From some other researches, medicinal mushrooms (*Agaricus bisporus*) are rich in specific  $\beta$ -glucans and chitin (dietary fibre) which may reduce serum LDL-cholesterol level by prohibiting cholesterol absorption and rising the faecal excretion of bile acids [22]. Oyster mushrooms (*P. sajor-caju*, *Pleurotus ostreatus* and *P. florida*) significantly corrected the level of different biochemical parameter and reduce the body weight in hypercholesterolemic rats [23]. Therefore, this study is the most specialized research to highlight the beneficial effects of mushroom consumption in hypercholesterolemia.

## 2 Materials and Methods

### 2.1. Location of the study

The research was performed in the laboratory of the Department of Anatomy and Histology, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh and the samples were also processed in the same laboratory.

### 2.2. Selection of experimental mice

Total thirty experimental Swiss albino mice with age of 3 weeks and weigh of about 20 to 22 grams were purchased from the Department of Pharmacy, Jahangirnagar University, Dhaka, Bangladesh.

### 2.3. Animal care and management

The mice were adapted at Animal Care Room in the Department of Anatomy and Histology, Bangladesh Agricultural University, Mymensingh-2202, for 7 days before being used for the experiment. In the research period the mice were supplied normal mice pellet with water to adapt the environmental condition and the experimental research laboratory was cleaned and washed on daily basis and proper hygienic and sanitary safety procedures were also taken (Figure 1).



**Figure 1.** Selection and proper management of experimental Swiss albino mice

### 2.4. Collection and preparation of mushroom powder

At first mushrooms (*Agaricus bisporus*) were collected from Horticulture Center, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh (Figure 2). Then mushrooms were cleaned and allowed them to dry completely for better dehydration. Mushrooms were sliced into thin slices and blender was filled with dehydrated mushrooms. Then blending was started until getting fine powder. Finally mushroom powder was stored in an air tight container for future use.



**Figure 2.** *Agaricus bisporus*

### 2.5. Study Design

The experimental Swiss albino mice were randomly divided into three (3) groups: control A, group B and group C; each having ten (10) mice. The control A group was fed with normal mice pellet and fresh water, group B was supplied with hypercholesterolemic diet (cow brain, 2g/kg body weight orally once daily) and group C was fed with hypercholesterolemic diet (cow brain, 2g/kg body weight/mice orally once daily) combined with mushroom powder (500g/kg body weight/mice) for 60 days.

### 2.6. Sample collection

After the experimental tenure (60 days), mice of each group were sacrificed ethically by doing chloroform anesthesia (**Figure 3**). Immediately after killing, the sample (liver) was collected in order to investigate the gross and histological study along with blood sample about 5ml was also collected from each mice by cardiac puncture following dissection for serum biochemical analysis. Finally hematoxylin and eosin (H&E) staining (liver sample) was done and examined under a light microscope.



**Figure 3.** Sample (blood) collection after chloroform anesthesia

## 2.7. Biochemical analysis

Total cholesterol (TC), triglyceride (TG) and high-density lipoprotein (HDL) cholesterol, were measured by using commercial kits (Asan Pharm Co Ltd, Seoul, Korea). The LDL cholesterol, were calculated using the following formula:

$$\text{LDL cholesterol} = \text{total cholesterol} - \text{HDL cholesterol} - (\text{triglyceride}/5)[24]$$

## 2.8. Statistical analysis

All the collected data were analysed by using Statistical Package for the Social Sciences (SPSS: version 20) software and reveal the results in tabular form. Statistical analysis was performed using one-way analysis of variance (ANOVA). Results were expressed as Mean  $\pm$  SD. Differences between groups were considered significant at  $**p < 0.01$  and  $*p < 0.05$  level.

## 2.9. Ethical approval

The present study and all experimental procedures were approved and performed according to the guidelines for the care and use of animals as established by Animal Welfare and Experimentation Ethics Committee, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh.

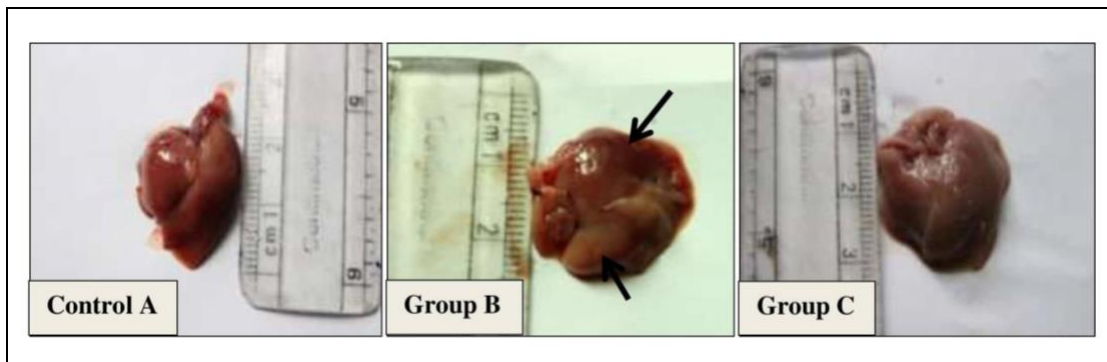
# 3 Results And Discussion

## 3.1. Weight of liver

In the gross study of liver, it was observed that the mean weight of liver changed in different groups. Mean weight of liver increased in hypercholesterolemic group B ( $1.832 \pm 0.049$  g) compare to control group A ( $1.758 \pm 0.022$  g) of mice due to hypercholesterolemic diet (**Figure 4**). On the other hand, mean weight of liver reduced in mushroom supplemented group B ( $1.756 \pm 0.027$  g).

## 3.2. Gross study

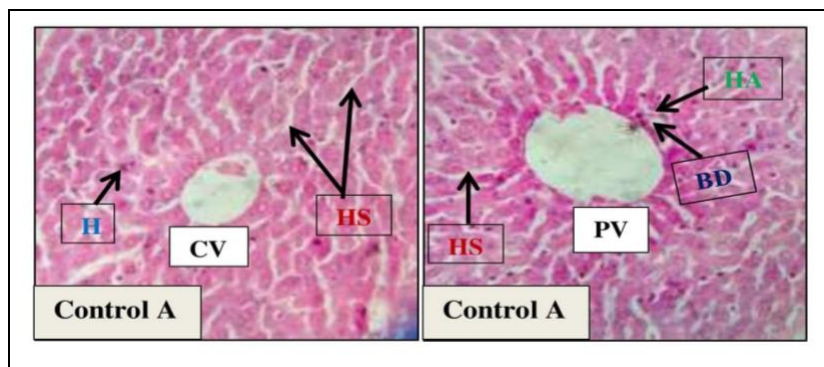
The results of the study showed that normal morphological appearance (Reddish, smooth, and shiny) of liver was found in the control group A of mice. Pale and hemorrhagic liver found in the mice of hypercholesterolemic group B (**Figure 4**). On the other hand, no significant change was observed in liver of mushroom supplemented group C.



**Figure 4.** Gross photograph of liver of control A, hypercholesterolemic group B and mushroom supplemented group C of mice. Liver became pale and showing hemorrhage (black arrow) in hypercholesterolemic group B of mice. Control A and mushroom supplemented group C showing normal gross morphology of liver.

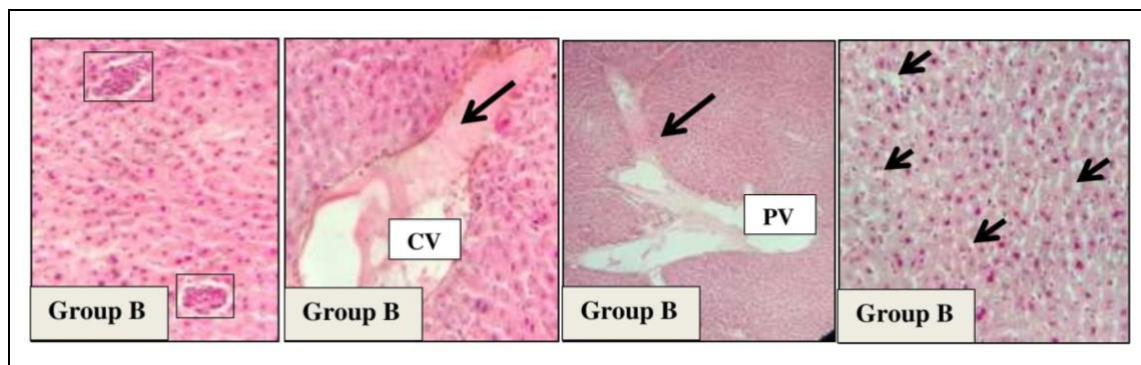
### 3.3. Histological study

Histological observation of liver showing dilation of central and portal vein, congestion in both portal and central vein, fat accumulation in hepatocyte and marked lymphocytic infiltration in hypercholesterolemic group B. But mushroom consumption prevents fat deposition in hepatocyte and lymphocytic infiltration. Only mild congestion and dilation of portal vein was found in mushroom supplemented group C (Figure 5-7).

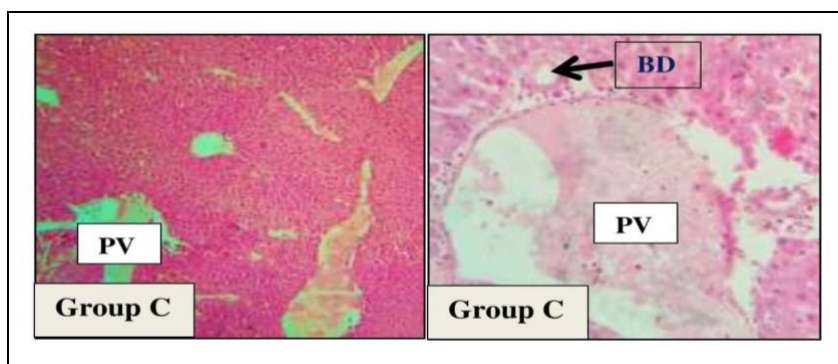


**Figure 5.** Photomicrographs of liver of control A group of mice showing normal central vein (CV) and portal vein (PV). CV= Central vein, PV=Portal vein, BD= Bile duct, HA=Hepatic artery, HS=Hepatic sinusoids, H= Hepatocytes. Images were photographed with a 40X objective (H&E stain).





**Figure 6.** Photomicrographs of liver of hypercholesterolemic group B of mice showing congestion and dilation in both central vein (CV) and portal vein (PV) (arrow), marked lymphocytic infiltration (rectangle) and fatty infiltration in the hepatocytes. Images were photographed with a 40X objective (H&E stain).

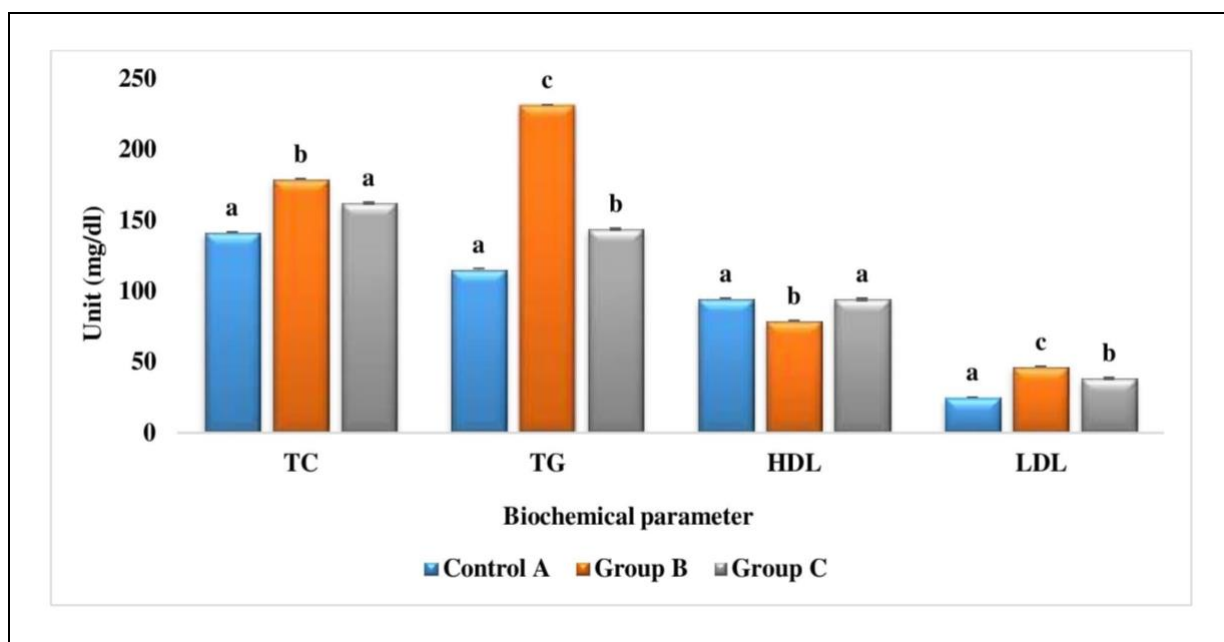


**Figure 7.** Photomicrographs of liver of mushroom supplemented group C of mice showing only mild congestion and dilation of portal vein. BD= Bile duct. Images were photographed with a 40X objective (H&E stain).

### 3.4. Lipid profile analysis

In the current experiment, the mean value of total cholesterol (TC) concentration increased by 27% in group B of mice compared to control A mice. Total cholesterol (TC) concentration decreased by 9% in group C compared to group B of mice (Table 2 and Figure 8). Triglyceride (TG) concentration increased by 72% in group B compared to control A mice. Triglyceride (TG) concentration decreased by 27% in group C compared to group B of mice (Table 2 and Figure 8). High density lipoprotein (HDL) level decreased by 31% and low density lipoprotein (LDL) level increased by 197% in group B mice compared to control A mice (Table 2 and Figure 8). High density lipoprotein (HDL) increased by 44% and low density lipoprotein (LDL) level decreased by 47% in group B compared to group C of mice (Table 2 and Figure 8).





**Figure 8.** Effects of mushroom feeding on lipid profile. Results are shown as Mean  $\pm$  SD. Bars with different letter (a, b and c) indicate significant differences at  $*p < 0.05$ .

**Table 2.** Effects of mushroom (*Agaricus bisporus*) feeding on serum lipid profile

Parameters (mg/dl)	Control (A)	Group (B)	Group (C)
TC	141.296 $\pm$ 0.318 <sup>a</sup>	179.044 $\pm$ 0.412 <sup>b</sup>	162.358 $\pm$ 0.537 <sup>a</sup>
TG	115.108 $\pm$ 1.067 <sup>a</sup>	231.294 $\pm$ 0.258 <sup>c</sup>	143.876 $\pm$ 0.535 <sup>b</sup>
HDL	94.481 $\pm$ 0.395 <sup>a</sup>	78.906 $\pm$ 0.417 <sup>b</sup>	94.410 $\pm$ 0.527 <sup>a</sup>
LDL	24.892 $\pm$ 0.273 <sup>a</sup>	46.544 $\pm$ 0.203 <sup>c</sup>	38.618 $\pm$ 0.522 <sup>b</sup>

Mushroom is being used as a traditional medicine for its antihyperglycemic and antihyperlipidemic potentials. *Agaricus bisporus* extracts or its bioactive compounds use as anti-cancer, antioxidant and anti-inflammation in all over the world as well as many human diseases such as diabetes mellitus, coronary heart diseases, fungal and bacterial infections, disorders of the many human immune system and cancers [25]. Due to the assessing of protein and fiber, mushrooms have also been found to be beneficial for weight loss [26]. This statement accurately reflect the gross observation of the present study where we found, mean weight of liver significantly increase in hypercholesterolemic group B, but in mushroom supplemented group C liver weight was about to similar as control group A. Hypercholesterolemic diet induces hepatic steatosis, pale and hemorrhagic liver. Hepatic steatosis mainly occurs due to the accumulation of fat droplets in hepatocytes. In the present research, pale and mild hemorrhagic liver was found in hypercholesterolemic group B. On the other hand, mushroom consumption in a regular basis helps to remain the structure of liver in normal like control group [1]. Edible

mushrooms not only reduce liver enzyme but also protect liver from fibrosis. Hypercholesterolemic diet causes dilation of central and portal vein, congestion in both portal and central vein, fat accumulation in hepatocyte and marked lymphocytic infiltration. Mainly due to the fat accumulation in hepatocytes as well as for congestion, central or portal vein become narrower or dilated [27,28,29]. Feeding of white button mushroom showed less fat accumulation in the cell of liver [30]. Eating of dried mushroom may protect the liver from congestion of central vein and infiltration with chronic inflammatory cells [31]. But sometimes mushroom supplementation may not recover the congestion of central vein (CV) with absence of fat droplets in hepatocyte [32]. Early investigation reports showed that mushroom intake is an effective strategy for obesity prevention because it reduced visceral fat accumulation [33]. In the present study, mushroom (*Agaricus bisporus*) consumption in diet reduced triglyceride (TG), total cholesterol (TC) and low density lipoprotein (LDL) level respectively by 9%, 27%, 47% and increased high density lipoprotein (HDL) level by 44% compared to hypercholesterolemic mice (**Table 2 and Figure 8**). Some of the researchers mentioned the similar findings as our current study that in hypercholesterolemic mice, oral feeding of *Agaricus bisporus* about 4 weeks resulted in a noticeable decrease in low-density lipoprotein (LDL) and plasma total cholesterol (TC) (33.1% and 22.8% respectively) ( $*p < 0.05$ ) and significant decrease in TG concentrations approximately by 20.8% ( $*p < 0.05$ ) [19]. Triglyceride (TG), total cholesterol (TC) and low-density lipoprotein (LDL) were significantly decreased ( $*p < 0.05$ ) along with HDL cholesterol was significantly increased ( $*p < 0.05$ ) by the 2% mushroom intake with daily feed compared to the control group [4]. Feeding of 5% oyster mushrooms (*Pleurotus sajor-caju*, *Pleurotus ostreatus*, and *Pleurotus florida*) powder reduced the plasma total cholesterol level by 21%, 37% and 16% respectively and reduced the triglyceride level by 24%, 45% and 14% respectively. LDL ratio also reduced by 45%, 64% and 41% for *Pleurotus sajor-caju*, *Pleurotus ostreatus* and *Pleurotus florida* fed rats, respectively. Our present findings are also similar with the other study that consumption of mushroom powder increases the excretion of total lipids and cholesterol through faecal matter that exhibited the hypocholesterolemic activities [34,35]. *Agaricus bisporus* contains 565.4 mg/kg of lovastatin and white button mushroom decrease the total serum cholesterol level [36]. Mushroom also contain both soluble and in soluble fibers; where the soluble fibre is mainly chitosans and beta-glucans polysaccharides that are the components of cell walls. This soluble fibre prevents and manages cardiovascular diseases.

## 5. Conclusion

Most of the people in our society don't have any idea about their daily diet. Lack of exercise and frequent consumption of fatty foods enormously develop various health complications. Our society fully depends on commercial drug for such type of health complications but they are not habituated with herbal medicine which is fully free from side effects and having early

recovering actions along with its cost effectiveness. From the present study, it can clearly be suggested that mushroom (*Agaricus bisporus*) has significant health benefits especially hepatoprotective as well as hypolipidemic actions. Further studies are also necessary to know how the mushroom abates the hypercholesterolemia along with recover the cellular alteration of hypercholesterolemic liver in mice.

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### **Conflict of interest**

We declare that we have no conflicts of interest to disclose.

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