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Mushroom Powder Ameliorates the Metabolic Syndromes by Maintaining Glucose and Lipid Homeostasis in High Sugar Diet-fed Mice

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Abstract

Diets high in added sugar both directly and indirectly promote the development of metabolic diseases such as diabetes and obesity. The objective of this study was to evaluate the effects of mushroom powder (MP) in preventing the development of metabolic disorders caused by a high sugar diet. Swiss albino male mice were fed with normal diet (ND) or high sugar diet (HSD) in supplementation with or without mushroom powder. The results showed that supplementation of MP in high sugar diet effectively reduced the food intake and body weight of mice. Moreover, MP sustained glucose tolerance in HSD-fed mice and a significantly lower glucose level was observed in the MP supplemented group than that of the HSD group. Furthermore, kidney and heart weight were almost similar among the groups. However, MP supplementation significantly reduced the liver weight in comparison to that of HSD control group. The wet weights of white adipose tissue and brown adipose tissue were also lower in the MP supplemented groups in comparison to that of HSD group. In addition, supplementation of MP in the high sugar diet significantly decreased the level of total cholesterol, triacylglycerides, and LDL content. In conclusion, these results suggest that mushroom powder was effective in preventing the development of diabetes and obesity induced by a high sugar diet. As mushroom powder is an abundant source of nutrients, therefore, this powder can be an important dietary supplement for treating metabolic disorders instigated by a high-sugar diet.

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Introduction

Diabetes mellitus is a metabolic disorder results in elevated blood sugar levels (hyperglycemia), abnormalities in insulin secretion or action at peripheral tissues, and reduced insulin sensitivity at skeletal muscle, adipose, and liver tissues. Insulin resistance is the result of this reduced insulin sensitivity (American Diabetes Association, 2009). Diabetes mellitus (DM) prevalence is a serious and expanding global health issue. Obesity and a sedentary lifestyle are significant environmental and genetic (heredity) contributors to Type 2 Diabetes.

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Overnutrition, insufficient exercise, and other factors can hasten the development of obesity, also known as excess body fat. Insulin resistance (IR) and metabolic abnormalities are caused by excessive energy intake and poor energy expenditure, which causes lipid accumulation in internal tissues such as adipose tissue, the liver, muscles, and other tissues and finally play a pivotal role to increase the incidence of diabetes and obesity in developing countries. According to the previous report by Barrière *et al.* (2018), consuming a high-sugar diet (HSD) hastens the onset of diabetes and obesity. However, South Asian nations choose to consume a variety of sugary meals that contain simple sugars and refined carbohydrates. That's why to counteract the negative effects of high sugar consumption, an alternate approach is required. Although there are a few restorative alternatives accessible for the management of diabetes, majority of them exhibit short to medium-term response. Further, most of current treatments are related with an expanded chance of antagonistic impacts such as gastrointestinal intolerance (metformin), weight gain (sulphonylureas, insulin and thiazolidinediones), hypoglycaemia (insulin and sulphonylureas), and myocardial dead tissue (rosiglita- zone) (Nathan *et al.,* 2009). As a result, there has been an increase in research on natural products and their active ingredients that have therapeutic potential for diabetes and obesity. Preventive measures for maintaining blood glucose homeostasis include maintaining a healthy body weight, eating low glycemic foods and soluble fibers, limiting refined sugars and trans fats, and engaging in other activities (Hod *et al*., 2015). Various plant parts have been reported to be high in fibers, vitamins, minerals, and phytonutrients, and to have beneficial effects in the treatment of diabetes, cardiovascular disease, obesity, and some cancers (Craig, 2010). Medicinal plants contain anti-diabetic compounds such as, phenolic compounds, flavonoids, alkaloids, and tannins that increase insulin secretion or decrease intestinal glucose absorption to improve pancreatic tissue efficiency (Kooti *et al*., 2016).

Mushroom is an excellent source of natural medicines with anti-diabetic activity. A mushroom is a type of macrofungus, and *Pleurotus* species are commonly referred to as oyster mushrooms. All across the world, these mushrooms are cultivated as food particularly in Southeast Asia, India, Europe, and Africa. People have cultivated and consumed mushrooms for hundreds of years due to their appealing sensory characteristics (Phat *et al.,* 2016), abundant nutritional compositions (Kalac *et al*., 2016), multiple functional activities (Roupas *et al.,* 2012), and manageable cultivation conditions. Edible Mushrooms are a high-quality source of protein, minerals, polysaccharides, unsaturated fatty acids, and secondary metabolites. Cultivated mushrooms are richer in protein and minerals, higher in vitamins B, D, and K, and lower in fat (Manzi *et al.,* 2001). In addition, the high dietary fiber content of mushrooms has been reported to act as an antitumor and antiviral agent (Zhang *et al.,* 2004a and 2004b). Moreover, mushrooms are known for being a good source of amino acids, which play an important role in their flavor (Mau *et al.,* 1998). Mushrooms contain bioactive components, particularly phenolic compounds and polysaccharides that have been shown to be effective antioxidants (Cheung, 2008, Li *et al*., 2017). Non-enzymatic molecules such as ascorbic acid and carotenoids have been found in mushrooms and act as antioxidants (Liu, 2004). Mushroom has been shown to increase antioxidant status in pancreatic, liver, and kidney tissues, all of which have significant hypoglycemic and hypolipidemic activities (Rony *et al.,* 2015). In STZ-induced diabetic rats, mushroom consumption was shown to lower blood glucose levels, improve serum lipid profile, liver function enzymes, liver glycogen, and enzymatic antioxidants activities in the liver, pancreas, and kidney (Jayasuriya *et al*., 2012). However, no study has yet been done to evaluate the beneficial effects of mushroom powder in mice on a high-sugar diet. Therefore, we carried out the present study to investigate whether supplementing with mushroom powder could help to halt the development of metabolic abnormalities induced by a high sugar diet (HSD).

Materials and Methods

Preparation of mushroom powder

Mushrooms (Oyster) were procured from the Horticulture Center, Mymensingh, Bangladesh. After collection, fresh mushrooms were cleaned, treated with $HgCl_2$, and then were cleaned once more. For eight to ten days, the mushrooms were dried in the shade at room temperature. After proper drying, the mushrooms were powdered with grinding machine and sieved. Before being used in the experiment, the powder was immediately placed in a sterilized jar and sealed.

Experimental animals

The Animal Resources Facility of International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR, B) provided six-week-old Swiss Albino male mice, which were acclimated to the new environment for 10 days. Animals were kept in a well-ventilated room at a temperature of 28°C with a relative humidity of 70–80%. Normal food and water were available *ad libitum* before the starting of feeding experiments. Animals were divided into four groups and each group contained at least 4 mice. The Animal Welfare and Experimentation Ethics Committee of Bangladesh Agricultural University approved (AWEEC/BAU/2021_16) all of the study's protocols.

Diet paradigms

Regular food preparation involves maize, groundnut cake, soybean, fish meal, oyster shell, salt, vitamin premix and rice bran at different proportion (Ulla *et al.,* 2017). Four types of food were formulated with supplementation of sucrose and/or mushroom powder. Types of food are as below-i) 100% Normal food formulation- Control group (ND); ii) 80% Normal food formulation + 20% Mushroom powder (MP); iii) 70% Normal food formulation + 30% sucrose (HSD); iv) 50% Normal food formulation + 30% sucrose (HSD) + 20% Mushroom powder (MP). The dose was justified with references to earlier investigations (Santoso *et al*., 2019, Li *et al*., 2016). To assure the quality of the animals' food, diets were changed daily. Six mice were assigned in each treatment group, and each mouse was housed in its own cage. The diet paradigm-based treatment was administered four consecutive weeks.

Measurement of food intake, water intake and body weight

The amount of food and water consumed by each mouse was measured weekly at 10:00 am for 4 weeks according to the following formula: Food / Water intake = Initial food / water weight- remaining food / water weight. The body weight of each mouse was also measured weekly utilizing an electric balance (eki300-2n electronic scale, A&D company Ltd., Korea) up to the end of the experiment.

Intraperitoneal glucose tolerance test

Following the protocol outlined in a previous study, the intraperitoneal glucose tolerance test (ipGTT) was performed at the end of the feeding treatment (Maejima *et al*., 2014). By moving mice to clean cages without food or waste in the bottom or hopper, mice were fasted for about 4 hours. At all times, access to drinking water was ensured. A scalpel blade that was either new or sterile was used to score the tip of the tail. The first small drop of blood was discarded. On the test strip of the blood glucose meter, a tiny drop of blood (<5µl) was applied. A standardized automated blood glucose test meter was used to measure

blood glucose level (Glucoleader TM Enhance Blood Glucose Meter, HMD Biomedical Inc., Hsinchu County, Taiwan). Each mouse received a single injection of glucose (2 g/kg BW) intraperitoneally. Each mouse's blood glucose level was measured at 15, 30, 60, and 120 minutes after receiving intraperitoneal glucose. The blood glucose levels in the ipGTT were then used to prepare the area under the curve (AUC) data.

Blood samples collection and preparation of serum

According to the previously stated procedure, at 28th day of the feeding experiment, after 18 hours of fasting, blood samples were taken from the Posterior Vena Cava (Hoff *et al*., 2000). The mice were put inside the airtight container that contained chloroform-soaked cotton. By making a V-cut through the skin and abdominal wall 1 cm caudal to the rib cage, the abdominal cavity of the anesthetized mouse was accessed. The liver was pulled forward while the intestines were moved to the left. Between the kidneys, the posterior vena cava's largest section was found. A 1 ml syringe and a 26-gauge needle were employed to collect the blood. The needle was carefully inserted into the vein and blood was drawn slowly until the vessel wall collapses. The blood-containing tubes were then centrifuged for 10 minutes at 4ºC at 4000 rpm (Gyrozen 1580R Multi-Purpose High-Speed Refrigerated Centrifuge, Gangnam-gu, Seoul, KOREA). After centrifugation, a fresh tube was filled with the supernatant serum that had been separated from the undesirable blood cells using a micropipette. Prior to the lipid profile assay, serum samples were kept at -20 ºC.

Measurement of organs weight

After collecting the blood samples, internal organs like the liver, heart, and kidney were harvested, and they were then trimmed to remove extra tissues. The organs were cleansed in a saline solution, and then the saline on the surface was removed by placing the organ on filter paper. After that, a digital balance was used to measure the organ weights (eki300-2n electronic scale, A&D company Ltd., Korea).

Determination of lipid profile parameters

Serum lipid profile studies includes the analysis of serum parameters such as total cholesterol (TC) level determined by CHOD-PAP method (Richmond, 1973); triglyceride (TG) level measured by GPO-PAP method (Cole *et al*., 1997); HDL cholesterol level estimated by CHOD-PAP method (Henry *et al*., 1974). HumaTex febrile antigen test kit (Human Diagnostic, Wiesbaden, Germany) was employed, and Humalyzer (Model No. 3000, Human GmbH, Wiesbaden, Germany) was used to calculate the absorbance of each test. The Friedewald equation was used to quantify serum LDL cholesterol values (Friedewald *et al*., 1972) as follows:

LDL cholesterol (mg/dl) = Total cholesterol– HDL cholesterol– (Triglyceride/5)

Statistical analysis

Using Prism 5, all statistical calculations were carried out (Graph Pad Software, CA). All information was presented as mean ± SEM. Tukey's post-hoc analysis was used after an analysis of variance (ANOVA) to justify the significant differences among the groups. The $p < 0.05$ was set as a significant value for all analyses.

Results and Discussion

Effect of mushroom powder on food intake of mice

Weekly food intake of each mouse was measured for the duration of the feeding experiment for 4 weeks. Food intake among the groups did not differ significantly before the start of the experiment (Fig. 1). However, incorporation of 30% sucrose and 20% mushroom powder into the food, exert an effect in the food intake per mouse from the first week of the treatment. Food intake was insignificantly higher in High sugar diet (HSD) supplemented group as compared with the control group (ND) for the duration of the experiment. MP supplementation reduced the food intake of ND-fed mice though it was not significant. Furthermore, supplementation of mushroom powder (MP) significantly reduced the food intake in comparison to HSD-fed group from 3 weeks of the feeding experiment. Moreover, food intake between (ND+MP) group and (HSD+MP) was comparable throughout the experimental period.

Effect of mushroom powder on water intake

We also measured the water intake throughout the experimental period. At the beginning of the experiment, water intake was comparable among the groups. However, 20% mushroom powder (MP)

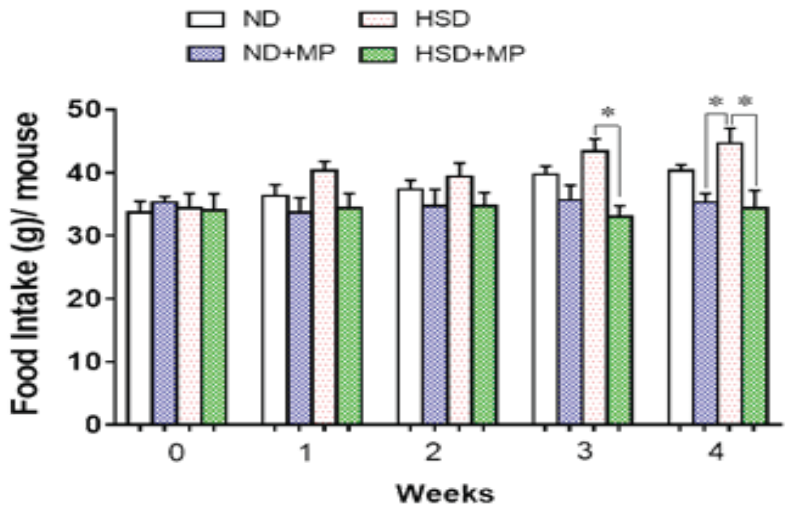

Fig. 1. HSD-induced hyperphagia was alleviated by MP supplementation. Average weekly food intake was measured for 4 weeks after the start of food supplementation. Bars represent mean ± SEM. n ≥ 3 for each group. *p<0.05 by ANOVA followed by Tukey's post-hoc test. ND: Normal Diet, HSD: High Sugar Diet, MP: Mushroom Powder.

supplemented groups showed a tendency to increase weekly water consumption in comparison to their control group though there was no statistically significant difference (Fig. 2).

Effect of mushroom powder on body weight of mice

The body weight of each mouse was measured to find out the efficacy of mushroom powder in reducing the development of HSD-induced obesity. Mushroom powder supplementation (20% weight basis) in ND

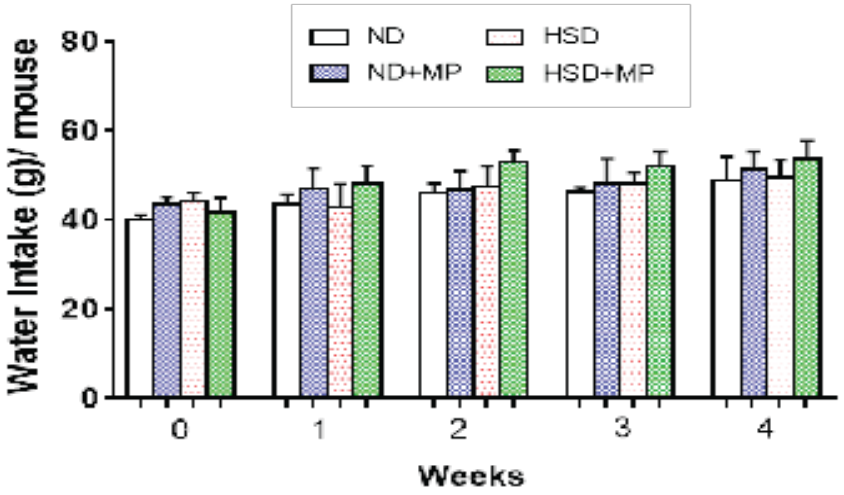

Fig. 2. Water consumption was unaffected by the supplementation of MP. Over the course of four weeks, water consumption per mouse was assessed every week. Bars represent mean ± SEM. n ≥ 3 for each group. ND: Normal Diet, HSD: High Sugar Diet, MP: Mushroom Powder.

diet-fed mice slightly reduced body weight which was not significant throughout the study period (Fig. 3). The results also showed that high sugar diet consumption increased body weight of the mice. However, mice fed with mushroom enriched diet significantly attenuated body weight as compared with HSD group at 3rd and 4th weeks of the experiment.

Effect of mushroom powder on intraperitoneal glucose tolerance

As we observed that MP supplementation effectively prevented the onset of diabetic symptoms such as obesity, hyperglycemia caused by HSD. We conducted ipGTT at the end of feeding experiment (at day 29)

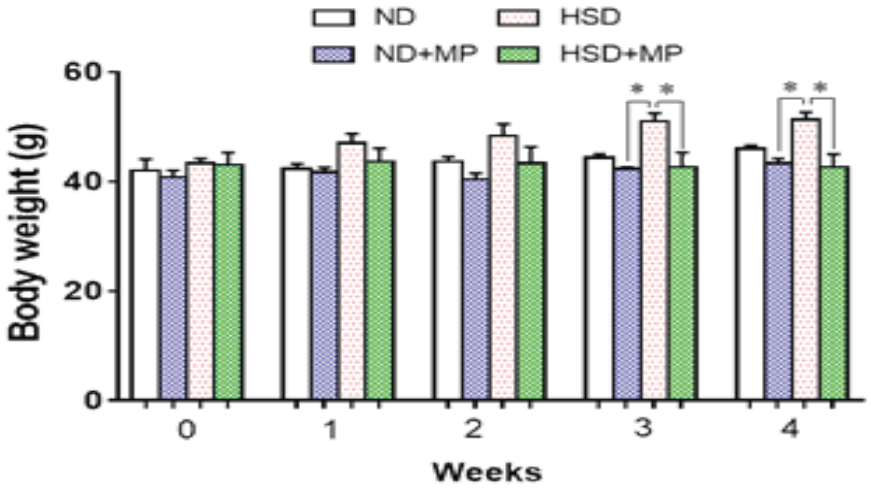

Fig. 3. MP counteracted the body weight gain in HSD-fed mice. The body weights of the mice were recorded on a weekly basis. Bars represent mean ± SEM. n ≥ 3 for each group. *p<0.05 by ANOVA followed by Tukey's post-hoc test. ND: Normal Diet, HSD: High Sugar Diet, MP: Mushroom Powder.

in order to evaluate the potentiality of MP to maintain the blood glucose homeostasis (Fig. 4A). High sugar diet supplementation slightly induced glucose intolerance as characterized by a sharp increase in blood glucose concentration after glucose challenge (i.p. 2g/kg BW). The GTT results also showed that, following an i.p. injection of 2 g glucose per kg BW, the blood glucose levels of the MP supplemented group tended to be lower than those of the HSD group almost at every time points (Fig. 4A). Furthermore, the AUC data (Fig. 4B) derived from the GTT graph depicted that the AUC of MP treated group was significantly lower as compared with the HSD group ($p < 0.05$).

Effect of mushroom powder on organs weight of mice

The liver weight of HSD fed mice showed a tendency to increase when compared to the control group,

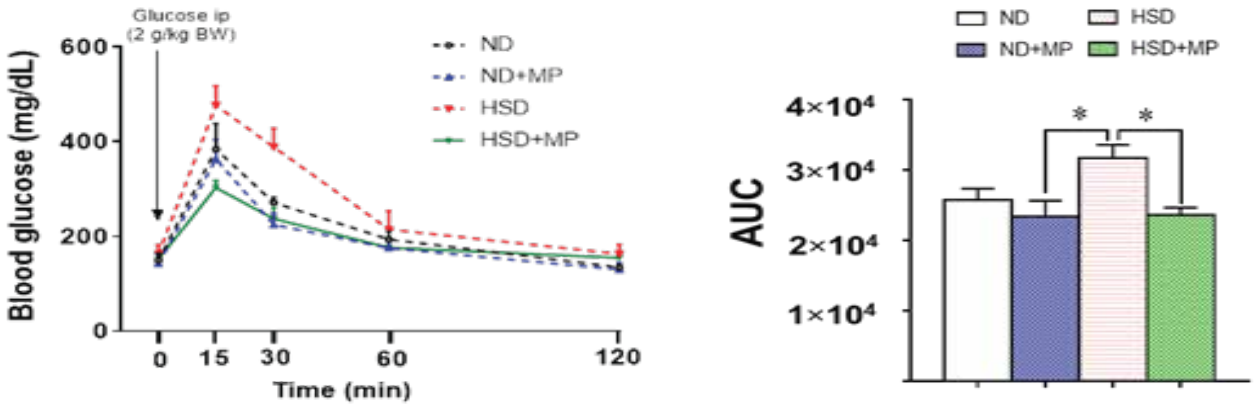

Fig. 4. MP sustained glucose tolerance in HSD-fed mice. (A) ipGTT was done at the end of the study after an intraperitoneal injection of glucose at a dose of 2 g/kg BW. (B) The corresponding area under the curve (AUC) values which showed significant difference about glucose utilization between HSD and HSD + MP fed groups. *p<0.05 by ANOVA followed by Tukey's post-hoc test. Bars represent mean ± SEM. n ≥ 3 for each group. ND: Normal Diet, HSD: High Sugar Diet, MP: Mushroom Powder.

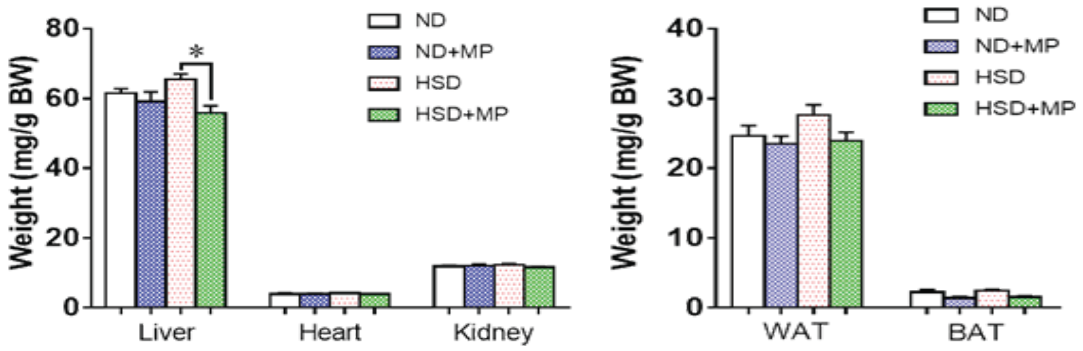

Fig. 5. MP treatment significantly attenuated the increased weight of liver in HSD-fed group. A) Organs (Liver, Heart and Kidney) weight, B) Weight of white adipose tissue (WAT) and brown adipose tissue (BAT) were measured at the end of the experiment after sacrificing the animals. *p<0.05 by ANOVA followed by Tukey's post-hoc test. Bars represent mean ± SEM. n ≥ 3 for each group. ND: Normal Diet, HSD: High Sugar Diet, MP: Mushroom Powder.

though the differences were very small (Fig. 5A). Mushroom powder (MP) supplementation (20%) attenuated the wet weight of the liver in the HSD-treated mice. Heart and kidney weights were comparable among the groups at the end of the study. The weight of white adipose tissue (WAT) and brown adipose tissue (BAT) were also affected by the diets. The HSD- treated mice had insignificantly higher amount of WAT and BAT than the control group. However, 20% supplementation of MP attenuated the wet weight of WAT and BAT of HSD-fed mice. The values for the weight of WAT and BAT were comparable among the ND, ND+MP, HSD and HSD+MP-fed groups (Fig. 5B).

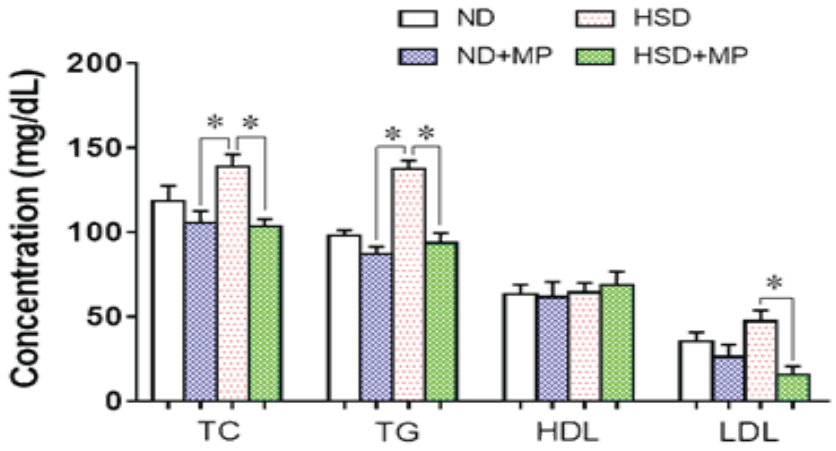

Fig. 6. MP treatment attenuated the HSD-induced elevation of serum concentrations of TC, TG and LDL. Blood lipid profile including total cholesterol (TC), triglycerides (TG), high-density lipoprotein (HDL-C) and low-density lipoprotein (LDL-C) were measured. *p<0.05 by ANOVA followed by Tukey's post-hoc test. Bars represent mean ± SEM. n ≥ 3 for each group. ND: Normal Diet, HSD: High Sugar Diet, MP: Mushroom powder.

Effect of mushroom powder on lipid profile parameters

High sugar diet (HSD) intake remarkably increased the total cholesterol (TC), triglycerides (TG) and low-density lipoprotein (LDL) concentration of the serum. Mushroom powder supplementation (20%) significantly reduced the serum concentration of the blood parameters (Fig. 6). There was no noticeable difference in serum HDL-cholesterol between the groups. Administration of MP in addition to normal diet showed an insignificant reduction in the serum concentration of TC, TG and LDL when compared with ND-fed mice.

The current investigation revealed that the supplementation of 20% mushroom powder (MP) with high sugar diet (HSD) significantly reduced the food intake and body weight gain in mice. The decrease in food intake observed in this experiment might be the result of either a decreased appetite or the diet that is less palatable. The chitin content in the mushroom powder may cause the decreased appetite. Fungal chitinous biopolymers (chitin and chitosan) are present in mushrooms. They have chitin in the cell walls (Wu *et al*., 2004), which makes up about 7% of their composition (Hassainia *et al*., 2018; Vetter, 2007). The deacetylated form of chitin, Chitosan, supplementation can cause a reduction in food intake in mice (Kumar *et al*., 2009). Food consumption may also be influenced by food palatability. The composition of food with additional plant-derived supplements that contain anti-nutritional components can have

an impact on its palatability (Dong & Pluske, 2007). Mushrooms contain compounds like tannins and phenolic acids (Yildiz *et al*., 2017), which may make them less palatable due to their bitter taste and cause the reduction in food intake (Dong & Pluske, 2007). Consuming high-sugar diets may cause excessive weight gain, which accelerates the development of obesity in rodents (Torres-Villalobos *et al*., 2015). According to earlier research, eating fast food and drinking beverages high in sugar can greatly raise the risk of obesity and diabetes in humans (Oo *et al*., 2017, El-Wakkad *et al*., 2012). Our present finding also demonstrated that HSD-fed mice showed a tendency to acquire body weight after 4 weeks of feeding treatment, and it was greatly inhibited by the 20% mushroom powder addition in their diet. A previous study also reported that a diet high in mushrooms could reduce body weight gain (Neyrinck *et al*., 2009)**.**

Consuming a high-sugar diet is linked to the development of metabolic dysregulations like diabetes and obesity (Lean and Morenga, 2016 & Barrière *et al*., 2018). One of the long-term metabolic disorders known as diabetes mellitus is characterized by elevated blood glucose concentration and associated with several disease states, especially cardiovascular disease. In this study, as expected, mice fed with HSD exhibited an increase in blood glucose concentration after glucose challenge (2g/kg BW). This finding may indicate to a dysfunctional blood glucose balance that could lead to the development of diabetes (Andrixopoulos *et al*., 2008). However, supplementation of MP in HSD significantly reduced the AUC in the high sugar diet-fed group. According to nutritional analysis by Rosli & Aishah (2012), mushroom powder (*Pleurotus sajor-caju*) has a high concentration of dietary fiber (35.6%), including *β*-glucan (3.57%). Moreover, dietary fiber and *β*-glucan have been scientifically demonstrated to have synergistic and protective benefits against a variety of ailments including heart disease, stroke, and diabetes, as they may help to reduce blood sugar and cholesterol levels in addition to other methods (Maier *et al.*, 2000). *β*-glucan is a soluble dietary fiber that can produce viscous solution, making it resistant to stomach digestive enzymes and delaying glucose absorption in the gut as well as gastric emptying rate (Chen & Raymond, 2008). Therefore, we can speculate that the dietary fiber present in mushroom and the ingredients present in it caused the delayed gastric emptying and may contribute to improve glucose tolerance in mice consuming high sugar diet. Apart from that, previous study also reported that oral administration of mushroom extract decreased serum glucose level in alloxan-induced diabetic mice (Badole *et al*., 2009).

Higher level of cholesterol and triglycerides are known as the risk factors for cardiovascular and metabolic diseases. In this experiment, supplementation of mushroom powder with high sugar diet showed a significant reduction in plasma cholesterol. One of the possible mechanisms is mushroom contains β-1, 3-D-glucan and pectin which bind to bile acids and inhibit the formation of cholesterol-bile micelle and resorption of cholesterol (Alam *et al*., 2009). Furthermore, mushrooms contain hypocholesterolaemic agent like mevnolin (monacolin K, lovastatin) (Gunde-Cimermann *et al*., 1993), which could reduce the rate-limiting enzyme's (3-hydroxy-3-methylglutaryl coenzyme A (HMGCoA) reductase) activity for cholesterol biosynthesis (Bobek *et al*., 1995). Furthermore, we observed that serum triglycerides were lower in mushroom powder supplemented group than that of HSD group. The characteristic high hemicellulose content in mushroom may influence the reduction of serum triglyceride level (Goyal, 2002). The presence of dietary fiber in mushroom may lead to triglyceride binding, which will boost fat excretion and limit the absorption of lipids (Zhang *et al.,* 1994). Jeong *et al.,* (2010) observed that lower weight of liver, lower liver cholesterol, and lower liver triglyceride levels were related with a reduction in plasma low-density lipoprotein cholesterol and an increase in high-density lipoprotein cholesterol. In our study, we also observed a significant reduction in the wet weight of liver. Moreover, we found no

noticeable changes between heart and kidney weights among the groups studied. There are a few studies that investigated the beneficial effect of mushroom powder on ameliorating HSD-induced fat deposition. This current study reveals that mice that received MP enrich diet had lower WAT than that of HSD supplemented group. Rats fed with a cholesterol diet also showed similar outcomes, particularly with the addition of 20% Maitake mushrooms resulting in a 43% reduction in fat deposition (Kubo *et al*., 1996). However, no significant difference was found in the weights of BAT among the groups. Further investigation is needed to completely understand detailed mechanism of potential benefits of mushroom powder to ameliorate the deleterious effect of high sugar diet consumption.

Mushroom powder could effectively maintain blood glucose homeostasis as well as body weight and food intake against the development of metabolic disorders such as diabetes and obesity caused by HSD in mice. As mushroom powder is an abundant source of nutrients, therefore, this powder could be an important dietary tool in the management of metabolic diseases.

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