THE IMPACT OF EDIBLE MUSHROOM SPECIES ON LIPID PROFILES AND BLOOD PICTURE OF MALE BALB-C MICE

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Abstract
Hyperlipidemia is currently one of the leading human health problems associated with blood lipid profile. This study was conducted to investigate the effects of two edible species of mushrooms: *Tremella fuciformis* and *Ganoderma* sp. as an alternative source of plant proteins in the micediet on lipid profile, blood biochemical traits (Glucose and Protein), and blood picture in BALB/c inbred mice. Blood was collected from heart-by-heart puncture to assay and calculate total cholesterol and lipoproteins (LDL, VLDL, HDL), and determine biochemical traits (Glucose and Protein), and the blood pictures. The results showed that the replaced mushroomswere found to decrease significantly (P≤0.05) levels of cholesterol and glucose in plasma. Likewise, low-density lipoprotein (LDL) and very low-density lipoprotein (VLDL) were significantly decreased with increasing ratios of mushrooms in the meal. The healthy cholesterol – HDL and Protein were increased significantly in T2 and T1 compared to the control treatment. Results also indicated that mice that fed edible mushrooms had significantly (P≤0.05) RBC, Hb higher than the control treatment. Findings of this work could approve that consumption of these two edible mushrooms may reduce the risk of hyperlipidemia and death rates associated with this health problem.

Keywords: Mushroom, cholesterol, hyperlipidemia, lipid profile, Balb-c mice

Abstract
高脂血症是目前与血脂谱相关的主要人类健康问题之一。本研究旨在研究两种可食用蘑菇：银耳和灵芝作为小鼠饮食中植物蛋白的替代来源对血脂、血液生化特征（葡萄糖和蛋白质）
Introduction

Cardiovascular disease is linked with high levels of mortality in many countries and associated with Hyperlipidemia, which refers to high levels of cholesterol in the bloodstream (Hu et al., 2013) commonly due to an unhealthy lifestyle including high cholesterol diets or regular saturated fatty acids consumption (Keong, 2015). As prediction by 2030, The World Health Organization (WHO) estimated more than three million people will be victims of cardiovascular disease (WHO, 2013). Also, a reduction of roughly 1% cholesterol in blood would reduce the risk of coronary heart disease 2-3% (Nguyen et al., 2007).

Since ancient eras, mushrooms have been utilized in folk medicine in almost world regions (Wasser and Weis, 1999). China and other Asian countries as earlier users have chiefly used mushrooms as traditional medicine. Mushrooms have been scientifically proven to be antineoplastic, antibacterial, hypoglycemic, antiviral, anti-inflammatory, hypocholesterolemic, and antioxidative properties (Guillamon et al., 2010; Wasser, 2011, 2014; Friedman, 2016). Various edible mushrooms are considered as an ideal food for the dietetic prevention of hyperlipidemia due to the high content of fiber, microelements, and their low-fat content (Cheung 1996). These were useful in synthesizing organic acids and enzymes (Zborowskiet al., 2003). In addition, they have significant roles in killing gram-positive pathogenic bacteria and increases normal flora (Gibson and Roberfroid, 1995), using as anti-tumor (Seljed, 1989), helpful in confirming humeral and cellular immunity (Ebihara and Schneement, 1989; Washburn and Hunter, 1998), and regulating fat and cholesterol in the blood (Washburn and Hunter, 1998). Furthermore, the edible mushrooms are so healthy when they are consumed by human and animals (Calilar et al. 2004; Gibson and Roberfroid, 1995; Gurbuz et al. 2004; Wiliam et al., 2004). So many species of mushrooms have been identified in Iraq. (Al Anbagi, 2014; 2021; Suliaman et al., 2017). Mushrooms are not only support healthy of their consumers, but also support healthy of their ecosystem as mycorrhizal fungi (Al Anbagi et al., 2019).

Based on the above facts, the aims of current study are to investigate the hypolipidemic effects of the edible mushrooms on the lipid profile [total cholesterol, low density lipoprotein (LDL), very low-density lipoprotein (VLDL), and high-density lipoprotein (HDL)] in mice after consuming their diets that have ratios of edible mushrooms and study the effects of edible mushrooms on blood picture and biochemical traits (protein, Glucose) in the mice blood plasma.
Materials and methods

Mushroom cultivation:

Two mushroom species were used in the present study including *Ganoderma* sp. and *Tremella fuciformis*. The mushroom, *Tremella fuciformis* was obtained from Juncao Research institutes, Fujian Agricultural and Forestry university, Fuzian, China, while *Ganoderma* fruits were collected from local fields, growing on Tamarisk tree (*Tamarixaphlly* L.). Production and growing techniques were referred to the Juncao Research Institute. Substrates was prepared by grinding wheat straw, wheat bran with corn cobs, pasteurized at 100°C for 8 hours, and incubated at 25°C in the plastic sheets for 30 days (Zhanxi, 2007). Spawn was prepared on cereal seeds following the same reference.

Animal care and feeding assays

The experiment was performed in animals’ laboratories belong to Veterinary College, Baghdad University. In this experiment, one hundred and eighty male balb-c (20-25 gm weight and three-month age) were tested in this experiment. Mice were obtained from the institute of the Plasma and Vaccines, Ministry of Health, Baghdad-Iraq. They were housed in stainless cages under controlled conditions at room temperature 25±5°C, humidity 60±5%, and 12 hours light cycle. The diet was grained into powder (40 mesh) by meat grinder, mixed with fresh water, modified to pellets (2 mm diameter) and dried in oven at 60°C. Previously, the mice were fed on commercial stock diet with fresh water for two weeks for adaptation. Then, *Ganoderma* sp., and *Tremella fuciformis* were used as sources of plant proteins in feeding of mice after preparation of mushroom powder. The mushroom fine powder of these species was added to the mice diet with and or without soybean in two concentrations 50% and 100% respectively.

Study Design and blood sample collection

The mice haphazardly divided into three groups based on mushroom concentrations. Each treatment consisted of sixty male mice. The control group (T0) was feed on the normal commercial diet. The second (T1) and third (T2) treatments (T1 and T2) were fed on commercial normal diet combined with mushroom powder at concentrations 50% and 100% respectively. The composition and concentrations of the commercial diet with replacement are shown in Table 1.

After eight weeks of feeding process, blood samples were collected from heart-by-heart puncture and were used for cholesterol, glucose, protein, and blood picture assays.

Table 1: Composition of the experimental diet.

<table>
<thead>
<tr>
<th>Comp/diet</th>
<th>Control</th>
<th>T1 (50%)</th>
<th>T2 (100%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Animal protein</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>cone</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Soybean meal</td>
<td>40</td>
<td>20</td>
<td>0</td>
</tr>
<tr>
<td>Mushroom powder</td>
<td>0</td>
<td>20</td>
<td>40</td>
</tr>
<tr>
<td>Yellow corn</td>
<td>15</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td>Barley</td>
<td>15</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td>Sunflower meal</td>
<td>15</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td>Vitamin mixture</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>0,5</td>
<td>0,5</td>
<td>0,5</td>
</tr>
</tbody>
</table>
Biochemical analysis of blood plasma
The blood samples were collected in heparinized tubes and separated by centrifuge (3000rpm) for 15 min. Plasma total cholesterol (TC), high density lipoprotein (HDL) levels were measured using enzymatic kit (Sigma). LDL cholesterol was calculated by the following equation:
LDL cholesterol = total cholesterol – HDL cholesterol -(triaclyglycerol/5).
Total cholesterol measured by the method described by Franey and Elias (1968), total protein determined by the method of Wotton (1964), glucose was assayed by using a method of Asatoor and king (1954).

Blood picture test
The samples were collected into a vascular containing heparin by heart vein puncture of 10 mice in each treatment, Hemoglobin concentration tested by varley etal (1980), totalRBC andWBC counted by method of Natt and Herrick (1952) and PCV percentage determined by method of Archer (1965).

Statistical Analysis
All the collected data were analyzed by using one-way ANOVA through SPSS software (Pallant, 2010). Statistical comparisons of means were measured using Duncan's multiple range test (Duncan, 1955).

Results and Discussion
Diet supplementation with two mushroom species significantly improved serum lipid profiles, comparable to the control treatment. Furthermore, the effect of edible mushrooms on the chemical and physiological traits of mice was observed (Figure1-10). Results showed that total cholesterol in plasma significantly decreased (P≤0.05) with increasing the ratios of edible Mushrooms in the diet supplementation (Figure 1). The maximal decrease of cholesterol values appeared at 100% mushroom replacement instead of soybean in the diet.

Findings indicated that all species of mushrooms cause significant effect (P≤0.05) on the low density of lipoprotein (LDL) levels, and very-low density lipoprotein (VLDL) (Figure2, 3). On the other hand, the good cholesterol (High-density lipoprotein) that had the lowest value in control was noted to be increased with increasing of the mushroom ratio in diets (Figure 4). Previous findings agreed with these results and revealed that a significant reduction in lipid profile, blood glucose, and liver marker enzymes of rats after nine days of oral feeding of the mushroom, *Ganoderma applanatum* was recorded (Hossain et al., 2021). Moreover, white button mushroom, (*Agaricus bisporus*), which is considered a valuable food for the treatment of hyperlipidemia, is a rich source of unsaturated fatty acids, protein, and fiber. It was successfully used to reduce triglyceride and serum total lipid in rats fed on 5 and 10% of this white button mushroom (Goyal & Grewal, 2017).

These results suggested that the decreased in total cholesterol, LDL, and VLDL may be referred to the high viscosity of edible mushrooms or the change in the viscosity of
intestinal mucosa of mice (Ebihara & Schneeman 1989). Furthermore, the decreased levels of total cholesterol may be also attributed to the activity of enzymes in *Lactobacillus* bacteria, which precipitate cholesterol and separate it from bile salt and couldn't be absorbed again (De Ross and Katan, 2000). On the other hand, hypercholesterolemia may be resulted from inhibition that occurred in cholesterol absorption with glycogen, which is presented abundantly in mushrooms. This decreased cholesterol may be due to increasing in bile acids (William et al., 2004), or decreasing of lipochrome that decreased total cholesterol. Feeding of *A. brasiliensis* to mice improved the serum lipid profile in hypercholesterolemic rats and impacts hepatic cholesterol metabolism via modulating the expression of key genes involved in this metabolism (de Miranda et al., 2017).

The increase of good cholesterol (HDL) in the mice, that fed edible mushrooms may be resulted from replacing HDL for LDL in the internal walls of veins and arteries (Nauck et al., 2000). Reduction in glucose level in mice blood was observed with increasing replacement of edible mushrooms compare to control after 8 weeks of feeding mice. Decreasing glucose concentrations could attribute to that glucose have important roles in recognizing the metabolism of fatty acids and triglycerides in the blood (Figure 5). Results in Figure 6 showed that the effect of edible mushroom in feeding ratios of mice caused a significant increase (p ≤ 0.01) in total protein. The results may indicate that the replacement of mushrooms instead of soybean led to increase in the immune response of mice. There were no significant differences in packed cell volume (PCV)% among all treatments compared to control (Figure 7) and the edible mushroom couldn't have any significant effects on white blood cells (Figure 8). Results in Figure 9, 10 indicated the mice that fed edible mushrooms had significant (P ≤ 0.05) RBC and Hb higher than the control treatment. These results suggest that the improvement of mice health agreed with a previous report revealed that mushroom affecting food for the medication of hyperlipidemia (Goyal & Grewal, 2017).

| Figure 1. | Cholesterol (mg/100ml) in blood of male Balb-C Mice after 8 weeks of feeding diets contain ratios of edible mushrooms. Control: only soybean as a source of plant protein without mushroom. T1: 50% soybean + 50% mushroom. T2: 0% soybean + 100% mushroom. Each value represents mean ± S.E. Different superscript letters in the same row are significantly different (p ≤ 0.05) according to Duncan test. |
| Figure 2. | Low-density lipoprotein (mg/100ml) in blood of male Balb-C mice after 8 weeks of |
feeding diets contain ratios of edible mushrooms. Control: only soybean as a source of plant protein without mushroom. T1: 50% soybean + 50% mushroom. T2: 0% soybean + 100% mushroom. Each value represents mean ± S.E. Different superscript letters in the same row are significantly different (p≤0.05) according to Duncan test.

Figure 3. Very-lowdensity lipoprotein (mg/100ml) in blood of male Balb-C mice after 8 weeks of feeding diets contain ratios of edible mushrooms. Control: only soybean as a source of plant protein without mushroom. T1: 50% soybean + 50% mushroom. T2: 0% soybean + 100% mushroom. Each value represents mean ± S.E. Different superscript letters in the same row are significantly different (p≤0.05) according to Duncan test.

Figure 4. High-density lipoprotein (mg/100ml) in blood of male Balb-C mice after 8 weeks of feeding diets contain ratios of edible mushrooms. Control: only soybean as a source of plant protein without mushroom. T1: 50% soybean + 50% mushroom. T2: 0% soybean + 100% mushroom. Each value represents mean ± S.E. Different superscript letters in the same row are significantly different (p≤0.05) according to Duncan test.

Figure 5. Glucose (mg/100ml) in blood of male Balb-C mice after 8 weeks of feeding diets contain ratios of edible mushrooms. Control: only soybean as a source of plant protein without mushroom. T1: 50% soybean + 50% mushroom. T2: 0% soybean + 100% mushroom. Each value represents mean ± S.E. Different superscript letters in the same row are significantly different (p≤0.05) according to Duncan test.

Figure 6. Protein (mg/100ml) in blood of male Balb-C mice after 8 weeks of feeding diets contain ratios of edible mushrooms. Control:
only Soybean as a source of plant protein without mushroom. T1: 50% soybean + 50%mushroom. T2: 0% soybean +100% mushroom. Each value represents mean ± S.E. Different superscript letters in the same row are significantly different (p≤0.05) according to Duncan test.

**Figure 7.** Packed cell volume (%) in blood of male Balbe-C ice after 8 weeks of feeding diets contain ratios of edible mushrooms. Control: only soybean as a source of plant protein without mushroom. T1: 50% soybean + 50%mushroom. T2: 0% soybean +100% mushroom. Each value represents mean ± S.E. Different superscript letters in the same row are significantly different (p≤0.05) according to Duncan test.

**Figure 8.** White blood cells (10$^3$/mm$^3$) in blood of male Balb-C mice after 8 weeks of feeding diets contain ratios of edible mushrooms. Control: only soybean as a source of plant protein without mushroom. T1: 50% soybean + 50%mushroom. T2: 0% soybean +100% mushroom. Each value represents mean ± S.E. Different superscript letters in the same row are significantly different (p≤0.05) according to Duncan test.

**Figure 9.** Red blood cells (10$^6$/mm$^3$) in blood of male Balb-C mice after 8 weeks of feeding diets contain ratios of edible mushrooms. Control: only soybean as a source of plant protein without mushroom. T1: 50% soybean + 50%mushroom. T2: 0% soybean +100% mushroom. Each value represents mean ± S.E. Different superscript letters in the same row are significantly different (p≤0.05) according to Duncan test.

**Figure 10.** Hemoglobin (gm/100ml) in blood of male Balb-C mice after 8 weeks of feeding diets contain ratios of edible mushrooms. Control: only soybean as a source of plant protein without mushroom. T1: 50% soybean + 50%mushroom. T2: 0% soybean +100% mushroom. Each value represents mean ± S.E. Different superscript letters in the same row are significantly different (p≤0.05) according to Duncan test.
letters in the same row are significantly different (p≤0.05) according to Duncan test.

**Conclusion**
The results of this study support the primary hypothesisthat edible mushroom significantly decreased the risks of the health problem, hyperlipidemia, which is determined via the levels of total cholesterol, low lipoprotein, and very low lipoprotein. On the other hand, the good healthy cholesterol (High density lipoproteins) that was increased related to increase the ratios of edible mushroom in the diet. The positive influence of mushrooms in this study and others clearly show the preventive action against hyperlipidemia on mice. However, further works are needed to study its effects in human beings.

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