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Influence of *Lentinus edodes* on Serum Enzyme Activity in Male Albino Wistar Rats

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ABSTRACT

In the 21st century, the use of natural resources in the food and medicine industries has emerged as a significant global focus, primarily due to their low toxicity and high specificity in modulating the immune system. Among these resources, mushrooms have been extensively studied for their diverse bioactive properties, including antibacterial, antifungal, antioxidant, antiviral, antitumor, cytostatic, immunosuppressive, antiallergic, antiatherogenic, hypoglycemic, anti-inflammatory, and hepatoprotective activities. This study aimed to investigate the effects of a diet supplemented with Lentinus edodes on the serum enzyme profiles of male albino Wistar rats. The results indicated that the group of rats receiving a diet with 10% L. edodes (Group D) exhibited significant alterations in their blood serum profiles compared to the control groups. These findings suggest that dietary supplementation with L. edodes may influence metabolic and immune responses in male albino Wistar rats, warranting further investigation into its therapeutic potential.

Keywords: *Antibacterial, Antifungal, Antioxidant, Antiviral, Antitumor, Cytostatic, Immunosuppressive, Antiallergic, Antiatherogenic, Hypoglycemic, Anti-inflammatory, and Hepatoprotective Activities.*

Introduction

Lentinus edodes, commonly known as shiitake mushroom, has garnered significant attention in recent years due to its remarkable health benefits and nutritional value. This edible fungus is not only a staple in culinary traditions but also a powerhouse of bioactive compounds that contribute to various pharmacological effects. Research has highlighted the potential of L. edodes in offering protective benefits against chronic diseases, including cancer, diabetes, and cardiovascular conditions. Its polysaccharides, particularly lentinan and schizophyllan, have been shown to possess potent anticancer properties, effectively inhibiting the growth of transplantable tumors in experimental models and enhancing survival rates [1]. Moreover, *L. edodes* exhibits significant antidiabetic effects, contributing to the regulation of blood sugar levels and insulin sensitivity. The mushroom's ability to lower blood pressure and cholesterol levels is particularly noteworthy, as hyperlipidemia is a well-documented risk factor for cardiovascular diseases [2]. Studies indicate that the polysaccharides derived from *L. edodes* can act as effective anticoagulants, further supporting heart health [3]. In addition to its pharmacological applications, L. edodes is nutritionally rich, containing high levels of protein, dietary fibers, and essential minerals, which contribute to its status as a functional food [4]. The comprehensive health benefits of *L. edodes* underscore its importance not only as a dietary staple but also as a potential therapeutic agent in preventive medicine. This review aims to consolidate the current understanding of the health-promoting effects of *L. edodes*, emphasizing its role in the prevention and management of chronic diseases through dietary and pharmacological interventions.

Materials and Methods

Preparation of Rat Feed

Normal Feed: The control group was fed a standard laboratory stock feed in pelleted form.

Normal Plus Mushroom Feed: To prepare the mushroom-supplemented diet, 100 g of the laboratory stock feed was finely powdered. Dried *Lentinus edodes* was then added in amounts of 2.5 g, 5 g, and 10 g, with each quantity being powdered separately. The mushroom powder was thoroughly mixed with the powdered lab stock feed using a small amount of hot water to enhance binding. The mixture was then shaped into pellets, air-dried, and stored in an airtight container at room temperature for future use.

Cholesterol-Rich Feed: For the cholesterol-rich diet, groundnut oil and egg yolk were incorporated into the normal feed to increase its cholesterol content.

Cholesterol Plus Mushroom Feed: To create the cholesterol-supplemented mushroom feed, 100 g of the cholesterol-rich feed was powdered. The previously powdered *L. edodes* was added in amounts of 2.5 g, 5 g, and 10 g, which were mixed thoroughly with the cholesterol feed using a small amount of hot water. This mixture was then formed into pellets, air-dried, and stored in an airtight container at room temperature.

Animals and Diets

Male Wistar rats, weighing approximately 100 g and aged five weeks, were utilized for this study. The rats were individually housed in wire mesh cages within a controlled environment at a temperature of 28 ± 2°C and a relative humidity of 50-60%. The lighting was set on a 12-hour light/dark cycle (lights on from 0600 to 1800 h), with air changes maintained at 10 to 12 per hour. Prior to the experiment, the animals were acclimated to the facility and provided with free access to water and a powdered laboratory stock diet. For the experimental groups, the rats received a diet consisting of 5% powdered *Lentinus edodes* mixed with the laboratory stock diet. The cholesterol group was given a diet enriched with oils, egg yolk, and groundnut to elevate serum cholesterol levels for experimental

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purposes. Ethical clearance for the study was obtained from the Institutional Animal Ethical Committee of Rajah Muthiah Medical College, Annamalai University, to ensure compliance with ethical standards for the use of male Wistar rats in research. The animals were managed following standard husbandry practices, and clinical observations, along with other relevant parameters, were recorded at intervals of 30, 60, and 90 days.

Experimental Design

The experimental rats were divided into the following groups:

- **Group A:** Rats fed with normal feed (control group)
- **Group B:** Rats fed with normal feed supplemented with 2.5% *Lentinus edodes*
- **Group C:** Rats fed with normal feed supplemented with 5% *L. edodes*
- **Group D:** Rats fed with normal feed supplemented with 10% *L. edodes*
- **Group E:** Rats fed with cholesterol-enriched feed
- **Group F:** Rats fed with cholesterol-enriched feed supplemented with 2.5% *L. edodes*
- **Group G:** Rats fed with cholesterol-enriched feed supplemented with 5% *L. edodes*
- **Group H:** Rats fed with cholesterol-enriched feed supplemented with 10% *L. edodes*

Each group was designed to evaluate the effects of varying concentrations of *L. edodes* in both normal and cholesterol-rich diets on the health and serum enzyme profiles of the rats

Clinical Symptoms and Body Weight

Both control and experimental groups of rats were weighed weekly to monitor changes in body weight throughout the study. Daily observations were made to assess any clinical symptoms or behavioral changes in the animals. Any noted symptoms were meticulously recorded to evaluate the overall health and well-being of the rats during the experimental period.

Assay of Aspartate Amino Transferase (AST) (Units/L)

Serum Aspartate Amino Transferase (AST) levels were measured using a diagnostic kit based on the method described by Reitman and Frankel (1957). In this assay, AST catalyzes the transfer of an amino group from L-aspartate to β-ketoglutarate, resulting in the formation of oxaloacetate and glutamate.

The amount of oxaloacetate produced was quantified by converting it into pyruvate through treatment with aniline citrate. The pyruvate was then reacted with 2,4-dinitrophenylhydrazine to form a 2,4-dinitrophenylhydrazone derivative, which exhibits a brown color in an alkaline medium. The absorbance of this hydrazone derivative was measured and correlated to AST activity. Results were expressed as International Units per Liter (IU/L) of serum.

Assay of Alanine Amino Transferase (ALT)

Serum Alanine Amino Transferase (ALT) levels were measured using a diagnostic kit based on the method described by Reitman and Frankel (1957). In this assay, ALT catalyzes the transfer of an amino group from L-alanine to β-ketoglutarate, resulting in the formation of pyruvate and glutamate. The pyruvate produced is then allowed to react with 2,4-dinitrophenylhydrazine, forming a 2,4-dinitrophenylhydrazone derivative, which exhibits a brown color in an alkaline medium. The absorbance of this hydrazone derivative is measured and correlated to ALT activity. Results were expressed as International Units per Liter (IU/L) of serum.

Assay of Alkaline Phosphatase (ALP)

Plasma Alkaline Phosphatase (ALP) levels were estimated using a diagnostic kit based on the method developed by Kind and King (1954). In this assay, ALP catalyzes the hydrolysis of disodium phenylphosphate into phenol and disodium hydrogen phosphate at a pH of 10.0. The phenol produced reacts with 4-aminoantipyrine in the presence of the oxidizing agent potassium ferricyanide, resulting in the formation of a red-colored complex. The absorbance of this complex is proportional to the enzyme activity and is expressed as International Units per Liter (IU/L) of serum.

Determination of Aspartate Amino Transferase (AST)

The results indicated that on the 30th day, the AST value in Group A, which was fed with normal rat feed, was 59.75 units/L. This group exhibited a gradual increase in AST levels over the observation period, reaching 62.09 units/L by day 90. Group D, which received a diet supplemented with 10% *Lentinus edodes*, recorded an AST value of 59.0 units/L on day 30, with a similar upward trend observed throughout the study, culminating in a value of 61.52 units/L by day 90. In contrast, Group E, fed with cholesterol-enriched feed, exhibited a higher AST level of 65.92 units/L on day 30. Group H, which received cholesterol feed supplemented with 10% *L. edodes*, recorded an AST value of 65.0 units/L on the same day. Notably, the AST values in Groups D and H displayed a decrease compared to the cholesterol group, indicating a potential beneficial effect of *L. edodes* supplementation on AST levels (Table 1).

Determination of Alanine Amino Transferase (ALT)

Overall, a reduction in ALT levels was observed in Group D (31.32 units/L) and Group H (39.25 units/L). The administration of a diet supplemented with *Lentinus edodes* to male Wistar rats resulted in a non-significant effect on ALT levels when compared to their respective control groups. Furthermore, rats fed with cholesterol-enriched diets exhibited higher ALT values compared to those on normal diets and those supplemented with mushrooms (Table 2).

Determination of Alkaline Phosphatase (ALP)

Throughout the observation period, an increase in ALP values was generally noted across all treatment groups. Both Group D (normal diet + 10% *Lentinus edodes*) and Group H (cholesterol diet + 10% *L. edodes*) exhibited no significant differences, showing statistically similar results to their respective control groups on all observation days (Table 3). Conversely, the rats fed with cholesterol-enriched diets consistently recorded significantly higher ALP values at each observation point.

Alkaline phosphatases (ALP) are a group of enzymes predominantly found in the liver (isoenzyme ALP-1) and bone (isoenzyme ALP-2). The measurement of ALP is clinically significant for assessing the potential presence of liver or bone disease. Elevated ALP levels can indicate liver congestion or obstruction, suggesting underlying liver dysfunction. In addition to liver conditions, an increase in AST, which is widely distributed in tissues, may signify non-specific tissue damage and is primarily associated with liver function. Elevations in AST can also suggest damage to the heart or muscle and may indicate deficiencies in certain hormones and vitamin E [5]. Overall, the serum enzyme profiles serve as important markers for liver and biliary tract dysfunction [4]. In this study, the co-administration of various levels of *L. edodes* resulted in a slight decrease in serum enzyme values compared to their respective control groups, suggesting a potential protective effect against enzyme elevation.

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Table 1 : Effect of L.edodes on the serum enzyme profile (AST) (units/L) of male albino wistar rats

Groups	30 days	60 days	90 days
Group A (Normal Feed)	59.75_b	60.27_a	62.09_b
Group B (2.5% *L.edodes*)	59.50_b	60.21_a	62.00_b
Group C (5% *L.edodes*)	59.21_a	60.11_a	61.82_a
Group D (10% *L.edodes*)	59.00_a	60.02_a	61.52_a
Group E (Cholesterol feed)	65.92_d	70.42_b	73.45_c
Group F (Cholesterol feed + 2.5% *L.edodes)*	65.25_c	70.38_b	73.32_c
Group G (Cholesterol feed + 5% *L.edodes)*	65.10_c	70.20_b	73.12_c
Group H (Cholesterol feed + 10% *L.edodes)*	65.00_c	70.10_c	73.05_c

Table 2: Effect of L.edodes on the serum enzyme profile (ALT) (units/L) of male albino wistar rats

Groups	30 days	60 days	90 days
Group A (Normal Feed)	32.21_b	35.31_a	41.92_b
Group B (2.5% *L.edodes*)	32.01_b	35.27_a	41.85_b
Group C (5% *L.edodes*)	31.96_b	35.13_a	41.70_b
Group D (10% *L.edodes*)	31.32_a	34.98_a	41.20_a
Group E (Cholesterol feed)	39.78_c	42.82_b	45.74_d
Group F (Cholesterol feed + 2.5% *L.edodes)*	39.72_c	42.78_b	45.71_d
Group G (Cholesterol feed + 5% *L.edodes)*	39.61_c	42.62_b	45.62_c
Group H (Cholesterol feed + 10% *L.edodes)*	39.25_c	42.41_b	45.42_c

Table 3. Effect of L.edodes on the serum enzyme profile (ALP) (units/L) of male albino wistar rats

Groups	30 days	60 days	90 days
Group A (Normal Feed)	118.82_a	120.72_a	127.81_a
Group B (2.5% *L.edodes*)	118.74_a	120.67_a	127.78_a
Group C (5% *L.edodes*)	118.61_a	120.58_a	127.65_a
Group D (10% *L.edodes*)	118.32_a	120.40_a	127.51_a
Group E (Cholesterol feed)	129.62_b	135.78_b	138.54_b
Group F (Cholesterol feed + 2.5% *L.edodes)*	129.51_b	135.61_b	138.48_b
Group G (Cholesterol feed + 5% *L.edodes)*	129.42_b	135.42_b	138.31_b
Group H (Cholesterol feed + 10% *L.edodes)*	129.12_b	135.10_b	138.10_b

Conclusion

The findings from this study demonstrate that the supplementation of *Lentinus edodes* in the diet of male albino Wistar rats has a notable influence on serum enzyme activity, specifically regarding Aspartate Amino Transferase (AST), Alanine Amino Transferase (ALT), and Alkaline Phosphatase (ALP). The results indicate that while *L. edodes* supplementation led to a decrease in AST and ALT levels compared to cholesterol-enriched diets, it produced no significant changes in ALP levels when compared to controls. The administration of *L. edodes* appears to offer a protective effect on liver function, as evidenced by the slight reduction in serum enzyme levels in the supplemented groups. This suggests potential benefits of incorporating *L. edodes* into the diet for promoting liver health and reducing enzyme elevation associated with dietary-induced stress. Further research is warranted to elucidate the mechanisms underlying these effects and to explore the therapeutic potential of *L. edodes* in mitigating liver dysfunction and enhancing overall health. These findings contribute to the growing body of evidence supporting the health benefits of mushroom supplementation in animal diets and may have implications for human health and nutrition.

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