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EFFECT OF DIETARY *LENTINUS EDODES* ON THE ERYTHROCYTE AND LEUCOCYTE COUNT OF MALE ALBINO WISTAR RATS

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Abstract

Mushrooms are rich in dietary fiber, minerals and vitamins and low in fat. The consumption of mushrooms or compounds present in mushroom extracts is suggested to have several health benefits. Globally, *Lentinus edodes* (shiitake) is the second most popular edible mushroom, its importance being attributed to both its nutritional value and medical applications. *L. edode*s is largely cultivated in China, Japan and other Asian countries because of its taste and nutritional values. The major objective of this research was to investigate the pharmacological effect of *Lentinus edodes*. The level of Red Blood Cells showed a steady increase in rats fed with normal feed or cholesterol feed along with various levels of mushroom supplementation similarly rats fed with various levels of mushroom supplementation showed a steady increase in the Level of White blood cells. Administration of various levels of mushroom feed in combination with normal feed showed an increase of haemoglobulin levels at all duration of time. Haemoglobulin level (13.21 g/dl) was recorded in Group D rats at the 90th day of observation.

***Key words*:** Shiitake, Male albino wistar rats and Haematology

Introduction

In the 21st century, the utilization of natural resources in food and medicine industries has become an international frontier due to their low toxicity and high specificity to activate immune system in body (Tsuchiya *et al*., 2003). Mushrooms are rich in dietary fiber, minerals and vitamins and low in fat (Manzi *et al*., 2001). The consumption of mushrooms or compounds present in mushroom extracts is suggested to have several health benefits. Globally, *Lentinus edodes* (shiitake) is the second most popular edible mushroom, its importance being attributed to both its nutritional value and medical applications (Hatvani, 2001). *L. edode*s is largely cultivated in China, Japan and other Asian countries because of its taste and nutritional values. *L. edodes* is revered in Asian medicine for its health-promoting effects, including antiviral, antifungal, antioxidant, and antitumor effects boosting the immune system, lowerind cholesterol, anticoagulant and cancer treatment (Watanabe, 2003). This mushroom has several health beneficial effects like anticancer, antidiabetic, hypotensive, hypocholesterolemic and antimicrobial activities (Khan *et al*., 2009). Also, it is important nutritionally because of its higher protein, dietary fibers and important mineral contents (Zhanhai yu *et al*., 2010)

Materials and Methods

Medicinal properties of *Lentinus edodes*

The medicinal properties of *L. edodes* were evaluated by testing the serum chemistry of *L. edodes* supplemented diet on male albino wistar rats. Accordingly, the serum Erythrocyte and Leucocyte count and haemoglobulin levels were among the parameters investigated.

Preparation of Rat Feed

Normal feed : lab stock feed in pelleted form.

Normal Plus Mushroom Feed: 100 g of lab stock feed in pelleted form was powdered. Then 2.5, 5 and 10 g of *L. edodes* was powdered and mixed thoroughly with the lab stock diet with the help of a little amount of hot water, and made into pellet form and air dried. Then it was stored in an air tight container at room temp.

Cholesterol Feed: Feed rich in cholesterol, *viz*., groundnut oil and egg yolk were mixed with normal feed and used.

Cholesterol Plus Mushroom Feed : Hundred g of cholesterol feed was powdered, then 2.5, 5 and 10 g of *L. edodes* was powdered and mixed thoroughly with the help of little amount of hot water, and made in to pellet form and then air dried. Then it was stored in an air tight container at room temp.

Animals and Diets

Male wistar rats weighing 100 g and five weeks old were used for the study. The rats were individually housed in wire mesh cages and kept in an isolated room at a controlled temp. of 28 ± 2°C and ambient relative humidity of 50-60 % on a 12-hour light: dark cycle (lights on from 0600 to 1800 h) and an air changes of 10 to 12 per hour. Animals were acclimated to the facility and given free access to water and the powdered laboratory stock diet. The animals belonging to experimental groups were given five per centage powdered *L. edodes* mixed with laboratory stock diet. For animals belonging to cholesterol group, oils, egg yolk and ground nut were mixed with normal feed to increase the serum cholesterol level for experimental

purpose. Necessary ethical clearance was obtained from Institutional Animal Ethical Committee of the Rajah Muthiah Medical College, Annamalai University to perform experimental studies on male wistar rats. The animals were reared with standard management practices and clinical as well as other parameters were recorded at 30 days, 60 days and 90 days duration.

Experimental Design

The experimental rats were grouped as

Group A	Rats fed with Normal Feed
Group B	Rats fed with Normal Feed + 2.5% *L. edodes*
Group C	Rats fed with Normal Feed + 5% *L. edodes*
Group D	Rats fed with Normal Feed + 10 % *L. edodes*
Group E	Rats fed with Cholesterol feed
Group F	Rats fed with Cholesterol feed + 2.5% *L. edodes*
Group G	Rats fed with Cholesterol feed + 5% *L. edodes*
Group H	Rats fed with Cholesterol feed + 10 % *L. edodes*

Clinical Symptoms and Body Weight

Both the controls as well as the experimental groups of rats were weighed at weekly intervals. The animals were observed daily for clinical symptoms if any and recorded.

Serum Chemistry

Serum profile was done by using ERBA CHEM semi auto analyzer. The values were taken on 30, 60 and 90th day of experiment.

Result and Discussion

Determination of Red Blood Cells (RBC)

Level of RBC showed (Table 1) a steady increase in rats fed with normal feed or cholesterol feed along with various levels of mushroom supplementation. The maximum RBC level of 7.97 g/l was recorded in Group D (10 per cent *L. edodes*) on the 90th day of observation when compared to control Group A which recorded 7.05g/l.The RBC level steadily increased in the duration of administering cholesterol feed plus mushroom diet in all groups. The maximum RBC level of 10.28g/l was recorded in Group H (cholesterol feed + 10 per cent *L. edodes*) on the 90th day of observation.

Determination of White Blood Cells (WBC)

Level of WBC showed (Table 2) a steady increase in rats fed with normal feed or cholesterol feed along with various levels of mushroom supplementation. The maximum WBC level of 10.08 g/l was recorded in Group D (10 per cent *L. edodes*) on the 90th day of observation. Group H (cholesterol feed + 10 per cent *L. edodes*) was recorded the maximum WBC level of 11.91 g/l n the 90th day of observation.

Determination of Haemoglobulin

Administration of various levels of mushroom feed in combination with normal feed showed (Table 3) an increase of haemoglobulin levels at all duration of time. Haemoglobulin level (13.21 g/dl) was recorded in Group D rats at the 90th day of observation. However, the supplementation of mushroom diet with cholesterol feed at various levels significantly increased the haemoglobulin levels at all the days of observation when compared to Group E. whereas Group H at 90th days of observation recorded the maximum haemoglobulin (15.98 g/dl)

The main function of the red blood cell (RBC or erythrocyte) is to carry oxygen from the lungs to the body tissues and to transfer carbon dioxide from the tissues to the lungs. This process is achieved by means of the Hb in the RBCs, which combines easily with oxygen and carbon dioxide and gives arterial blood a bright red appearance. To enable use of the maximal amount of Hb, the RBC is shaped like a biconcave disk; this affords more surface area for the Hb to combine with oxygen. The cell is also able to change its shape when necessary to allow for passage through the smaller capillaries. The RBC test, an important measurement in the evaluation of anemia or polycythemia, determines the total number of erythrocytes in a microliter (cubic millimeter) of blood (Aiko watnable *et al.,* 2006)

Haemaglobin, the main component of erythrocytes, serves as the vehicle for the transportation of oxygen and carbon dioxide. It is composed of amino acids that form a single protein called globin, and a compound called heme, which contains iron atoms and the red pigment porphyrin. It is the iron pigment that combines readily with oxygen and gives blood its characteristic red color. Each gram of Hb can carry 1.34 mL of oxygen per 100 mL of blood. The oxygen-combining capacity of the blood is directly proportional to the Hb concentration rather than to the RBC because some RBCs contain more Hb than others. This is why Hb determinations are important in the evaluation of anemia (Enman *et al*., 2007).

The Hb determination is used to screen for disease associated with anemia, to determine the severity of anemia, to monitor the response to treatment for anemia, and to evaluate polycythemia. Hb also serves as an important buffer in the extracellular fluid. In tissue, the oxygen concentration is lower and the carbon dioxide level and hydrogen ion concentration are higher. At a lower pH, more oxygen dissociates from Hb. The unoxygenated Hb binds to hydrogen ion, thereby raising the pH. As carbon dioxide diffuses into the RBC, carbonic anhydrase converts carbon dioxide to bicarbonate and protons. As the protons are bound to

Hb, the bicarbonate ions leave the cell. For every bicarbonate ion leaving the cell, a chloride ion enters. The efficiency of this buffer system depends on the ability of the lungs and kidneys to eliminate, respectively carbon dioxide and bicarbonate (Frank *et al*., 2006; Fukushima *et al*., 2009) stated that the rats that had been treated with *L. edodes* given orally or intraperitoneally, increased the leucocytes and haemoglobin content than the untreated rats. Yasuko *et al*. (2010) reported that the oral administration of *L. edodes* significantly changed the total erythrocyte count (RBC), total leucocytes count (WBC) and Haemoglobin content in the experimental rats. Thus the present findings show that the food supplementation with *L. edodes* could able to increase the haematological parameters.

Table 1: Effect of *L. edodes* on the Erythrocyte count (or) Red blood cells (RBC) (x 10^6 /µl) level in the serum of male albino wistar rats.

Groups	30 days	60 days	90 days
Group A (Normal Feed)	6.12_h	6.15_h	7.05_g
Group B (2.5% *L. edodes*)	6.28_g	6.29_g	7.28_f
Group C (5% *L. edodes*)	6.52_f	6.58_f	7.64_e
Group D (10% *L. edodes*)	6.92_e	6.98_e	7.97_d
Group E (Cholesterol feed)	6.05_d	6.10_d	6.14_d
Group F (Cholesterol feed + 2.5% *L. edodes)*	7.82_c	8.42_c	9.92_c
Group G (Cholesterol feed + 5% *L. edodes)*	8.05_b	8.87_b	10.15_b
Group H (Cholesterol feed + 10% *L. edodes)*	8.18_a	9.10_a	10.28_a

Table 2 : Effect of *L. edodes* on the Leucocyte count (or) White blood cells (WBC) (x 10^6 /µl) level in the serum of male albino wistar rats

Groups	30 days	60 days	90 days
Group A (Normal Feed)	8.51_h	8.62_h	9.25_h
Group B (2.5% *L. edodes*)	8.74_g	8.91_g	9.42_g
Group C (5% *L. edodes*)	8.91_f	9.10_f	9.74_f
Group D (10% *L. edodes*)	9.18_e	9.21_e	10.08_e
Group E (Cholesterol feed)	8.45_d	8.49_d	8.50_d
Group F (Cholesterol feed + 2.5% *L. edodes)*	10.21_c	10.45_c	11.28_c
Group G (Cholesterol feed + 5% *L. edodes)*	10.48_b	10.72_b	11.58_b
Group H (Cholesterol feed + 10% *L. edodes)*	10.71_a	11.04a	11.91_a

Table 3: Effect of *L. edodes* on the Haemoglobin (g/dL) level in the serum of male albino wistar rats

Groups	30 days	60 days	90 days
Group A (Normal Feed)	11.62_h	11.75_h	12.21_h
Group B (2.5% *L. edodes*)	11.78_g	11.98_g	12.51_g
Group C (5% *L. edodes*)	11.92_f	12.10_f	12.92_f
Group D (10% *L. edodes*)	12.12_e	12.28_e	13.21_e
Group E (Cholesterol feed)	11.53_d	11.58_d	11.60_d
Group F (Cholesterol feed + 2.5% *L. edodes)*	12.32_c	14.10_c	15.28_c
Group G (Cholesterol feed + 5% *L. edodes)*	12.59_b	14.52_b	15.52_b
Group H (Cholesterol feed + 10% *L. edodes)*	12.74_a	14.98_a	15.98_a

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