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Research Article

Preventive Effects of the Mushroom (*Volvariella volvacea*) on Leukocyte Parameters, Albumin and Alanine Aminotransferase in Wistar Rat on a High-fat Diet

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Abstract

Consumers are now showing a strong interest in bioactive foods such as mushrooms, which have beneficial effects on human health and reduce the risk of diet-related diseases such as obesity. This study aimed to evaluate the effects of dietary supplementation with powdered *Volvariella volvacea* (Vv) mushroom on leukocyte parameters, albumin and alanine aminotransferase to the Wistar rats induced rats on a high-fat diet for 42 days. Rats weighing an average of 96.2 ±1.3 g were fed control diets and diets based on mushroom powder at different percentages: negative control diet or distilled water (DW), positive control diet or high-fat diet (HFD), 5% diet (Vv 5% and HFD), and 10% diet (Vv 10% and HFD). Blood samples were collected from the animals at the beginning of the experiment (day 1) and again on day 42. Leukocyte parameters were measured using a BC-30s hematology analyzer, and albumin and alanine aminotransferase levels were measured using an automated spectrophotometer. The results showed that the consumption of 10% Vv mushrooms slowed the increase in the rate of WBC (20.8%) unlike the positive control group (53.1%). Neutrophil and lymphocyte counts were lower than in the positive control group (15.9% vs. 57.5% and 12.78% vs. 61.35%, respectively) with the 10% Vv dose, which is thought to be related to the overall immune response of animals fed this mushroom. Significantly reduced levels ($P < 0.05$) of the liver enzyme ALT were observed in the Vv 10% diet (16.50%) compared to the fat diet (68.50%), which helps prevent hepatic steatosis. Albumin levels, which increased significantly ($p < 0.05$) in the Vv 10% group (15.80%) and decreased significantly in the positive control group (16.95%), were influenced by protein intake. In summary, leukocyte parameters, the liver enzyme alanine aminotransferase and albumin levels were close to those of non-obese rats; therefore, Vv consumption could have benefits for human health.

Keywords

Mushroom *Volvariella volvacea*, High Fatty Diet, Leukocytes, Albumin, Wistar Rats

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1. Introduction

Blood is a highly specialized tissue composed of several types of cells and plasma components. White blood cells are cellular components that can be found not only in the blood but also in lymphatic tissues. Although they represent only a small percentage of blood volume, typically around 1% in a healthy individual, they constitute the immune system's first line of defense against foreign invaders [1].

V. volvacea, also known as the rice straw mushroom, is a type of edible mushroom with a tropical and subtropical distribution worldwide. [2] It belongs to the family Pluteaceae in the order Agaricales, subdivision Basidiomycetes [3].

In sub-Saharan Africa, particularly in Cote d'Ivoire, ethnomycological surveys have shown that local populations possess a good level of knowledge about medicinal mushrooms, notably the mushroom *Volvariella volvacea*. The survey results indicated that these populations use this species to treat malaria, diabetes, high blood pressure, and heart ailments. The fruiting bodies are cooked into a decoction, which is then drunk by the patient [3]. Edible mushrooms are thus a source of therapeutic agents beneficial to health. Thus, it has been reported that bioactive compounds such as β-glucan, selenium , flammutoxin, and volvatoxin found in V. volvacea exhibit antioxidant and antitumor activities, while interleukin-1β, interleukin-8, and tumor necrosis factor-α, also present in V. volvacea, possess anti-inflammatory and anticancer properties [4]. Similarly, essential amino acids in V. volvacea such as leucine, isoleucine, and phenylalanine, as well as vitamin B2, have been linked to the anti-inflammatory response [4].

Other medicinal properties have been attributed to mushrooms, including the inhibition of platelet aggregation [5, 6]. For example, the methanolic extract of the mushroom *Pleurotus florida* at 500 µg/mL showed significant inhibitory activity against adenosine 5′-diphosphate-induced human platelet aggregation [5]. Furthermore, blood morphology studies have shown that shiitake mushroom supplementation has an effect on white blood cell count, hemoglobin, and hematocrit levels [7]. However, few studies have been conducted on African mushrooms [8].

The objective of this work is to study the preventive effects of the mushroom (*Volvariella volvacea)* on leukocyte parameters, albumin and urea in the rats Wistar are fed a high-fat diet.

2. Materials and Methods

2.1. Animal Material and Livestock

The experimental protocol consisted of 32 rats mixed in 4 groups of 8, as well as *Rattus norvegicus* of Wistar strain composed of males, of the pet shop of the Physiology, Pharmacology and Pharmacopoeia laboratory of Nangui Abrogoua University, Abidjan. Rats aged 8 to 9 weeks and with a homogeneous mean body weight of 114.02 ±4.2 g are fed with standard diet (Ivograin®, Abidjan, Cote d'Ivoire) for negative control rat (DW) and standard diet, 30% pig fat for positive control rat (HFD) [9]. The last one was added *Volvariella volvacea* (5%Vv and 10%Vv) for experimental rats during 7 weeks (42 days). The breeding is done in a lighted room 12 hours a day, and whose temperature is kept constant (22 to 23 ℃) in an enclosure equipped with an air conditioner (Smart, Canada). The animals have free access to food and water and are weighed once a week.

2.2. Preparation of the Aqueous Extract of *Volvariella volvacea* (Vv)

The aqueous extract of Vv was prepared using the slightly modified traditional extraction methods of Daoudi and Kre [10, 11]. The *Volvariella volvacea* (Figure 1) samples were washed and then dried in thin layers away from sunlight for 25 days. Once dried, the mushroom material was ground. For decoction extraction, 100 ml of water was added to 10 g of powdered fungal material and boiled for 15 minutes, then filtered through filter paper and Wattman filter paper before being dried in an oven at 46 ℃ for 24 hours. The brown-colored dried *Volvariella volvacea* evaporates were collected and stored in sterile boxes to be incorporated into the diet of Wistar rats.

Figure 1. *Volvariella volvacea (Pluteacea).*

2.3. Blood Sampling

Blood samples were taken in healthy rats on day 1 and apparently healthy at the experiment end. The rats are anesthetized with a solution of ether soaked in cotton after 12 hours of fasting. The blood collected by punctures of the retro-orbital sinus is recovered in EDTA tubes in order to count the number and see the shape of the blood cells on the same day of sampling.

2.4. Determination of Leukocyte Parameters, Albumin and Alanine Aminotransferase (ALT)

This was performed directly by the complete blood count on days 1 and 42 to avoid cell autolysis and to obtain reliable results using the automated hematology analyzer (Sysmex X-1000, Japan). The complete blood count essentially provides information on the total number of leuko cells, neutrophils, basophils and lymphocytes [1]. To this were added albumin and ALT by an automated spectrophotometer [Randox, France].

3. Results

3.1. Variation in Leukocyte Parameters (WBC, Neutrophils, Lymphocytes, Basophils) from the 1st to Day 42

At day 42, a significant increase in the number of leukocytes in general was recorded in rats that received single pork fat as a supplement compared to the rest of the animals. In contrast, the basophil values did not register any significant variation in the different lots (Table 1).

Table 1. *Variation of certain leukocyte parameters in rats after Volvariella volvacea (Vv) eaten.*

	White blood cells (10^3 µl)		Basophils (10^3 µl)		Neutrophils (10^3 µl)		Lymphocytes (10^3 µl)	
	D1	J42	D1	J42	D1	J42	D1	J42
DW	11.6 ±1.1	13.1 ±1.2	0.35 ±0.02	0.43 ±1.50	2.06 ±0.21	3.07 ±0.8	9.7 ±0.1	12.7 ±0.8
FF	9.21 ±0.15	14.1±0.1 (53.1%)	0.81±0.07	1.03 ±0.01	3.2 ±0.5	6.04 ±0.01 (57.5%)	8.12±0.8	13.1 ±0.4 (61.35%)
D- 5%	12.16 ±1.2	15.1 ±1.30	0.6 ±0.03	0.8 ±0.10	2.15 ±0.6	4.82 ±0.4	9.07 ±0.38	11.04 ±0.1
D-10%	10.1 ±1.26	13.2 ±0.8 (20.8%)*	0.81 ±0.07	0.93 ±0.4	2.76 ±0.6	3.20 ±0.1 (15.9%)*	10.2 ±0.3	11.48 ±0.5 (12.7%)*

* p < 0.05: Comparison with the positive control group (CG).
DW: Distilled water; FF: Fatty food; J1: Day 1; J42: Day 42
D-5%: Incorporation rate of *Volvariella volvacea* at 5%
D-10%: Incorporation rate of *Volvariella volvacea* at 10%

3.2. Evolution Albumin and Alanine Aminotransferase Levels

Table 2. *Variation in albumin (Alb) and alanine aminotransferase (ALT) levels in rats.*

	Alb (g/l)		ALT (IU/L)	
	D1	J42	D1	J42
DW	23.04 ±1.6	28.2 ±1.05	73.15 ±0.4	73.12 ±0.3
HFD-30%	26.2 ±0.8	22.4 ±1.07 (16.95%)	77.2 ±0.8	130.1 ±0.5 (68.50%)
D-5%	22 ±0.67	25.1 ±1.1	69.4 ±0.11	85.4 ±1.2
D-10%	24.06 ±1.6	27.8 ±0.2 (15.80%)*	71.6 ±0.2	83.10 ±0.1 (16.50%)*

* p < 0.05: Significant difference of rats consuming Vv (5% and 10%) compared to the positive control (FF), n = 8, M ±ESM.
DW: Distilled water; HFD: High fat diet; J1: Day 1; J42: Day 42
D-5%: Incorporation rate of *Volvariella volvacea* at 5%
D-10%: Incorporation rate of *Volvariella volvacea* at 10%

4. Discussion

The development of obesity linked to a high-fat diet is of major importance and it has been shown response of the body's defense cells, including white blood cells, lymphocytes, neutrophils and basophils.

Consumption of the Vv mushroom by fat-induced animals prevented an increase in white blood cell, neutrophil, and lymphocyte counts compared to the positive control (20.8% vs 53.1%; 15.9%vs 57.5%; 12.78% vs 61.35%), respectively; this finding was more pronounced with the 10% Vv dose (Table 1). A slight increase in white blood cell count could be related to excess fatty acids in the blood. Indeed, leukocytes (white blood cells) primarily react by attempting to combat damaged cells or associated inflammatory processes, often leading to an increase in their number (leukocytosis) in response to inflammation or a resulting infection [12]. Furthermore, to avoid a chronic excess of fatty acids that can also lead to immune dysfunctions, bioactive components from Vv such as vitamins, trace elements, flavonoids and saponins also played a key role in balancing the redox state of white blood cells, thus protecting them from oxidative stress [13]. These processes are essential for the survival of the cell population in general, hence the slight increase observed in the number of WBCs in animals that received 10% Vv.

In the present study, the results showed a slight increase in neutrophil counts (neutrophils being a subpopulation of leukocytes) in Vv rats (Table 1) in the presence of excess fatty acids. These cells, which are among the first responders recruited to a high-fat diet, are part of the cell-mediated immune responses responsible for innate immunity, the first line of defense that helps eliminate foreign bodies and repair damaged tissues [14, 15]. In another study, extracts of V. *volvacea*, as a potential anti-inflammatory and anticancer agent, regulated (decreased) IL-8 expression in the activation of neutrophils as a host cell defense mechanism [16].

There was no significant variation ($p > 0.05$) in basophils between rats treated with the *Volvariella volvacea* doses and controls, although this mushroom is essential for the growth, differentiation, and proliferation of immune system cells, including basophils. Similarly, a fungal immunomodulatory protein (FIP) isolated from *V. volvacea* in mice promoted the maturation and function of dendritic cells, suggesting that this protein could have benefits for human health by enhancing immunity [17].

Lymphocytes represent up to 10% of non-adipose cells in adipose tissue and include T lymphocytes, B lymphocytes, NK cells and ILC2 cells [18]. The slight increase in lymphocytes in this study shows that the Vv mushroom inhibited, on the one hand, the development of obesity linked to dietary supplementation with a very high fat content (30%), and on the other hand, the recruitment of pathogenic B lymphocytes that were unable to accumulate in adipose tissue to produce chemokines that should activate T lymphocytes [19].

ALT levels (Table 2), they were significantly reduced ($p < 0.05$) compared to the high-fat diet, implying that administration of the 10%V mushroom would be beneficial in preventing hepatic steatosis by lowering the level of this transaminase. This result is consistent with those reported by Farhana [20] and Lotty [21]. This latter author found low levels of ALT (36.73U/ml) in obese rats consuming 20% mushrooms in their diet compared to the group of positive control rats (66.83U/ml) [21]. Similarly, ALT levels, like other transaminases, were reduced in rats fed a high-fat diet and treated with 2.5% *Ganoderma lucidum mushroom powder* (81.04U/l) compared to the positive control group (105.1 U/l), thus reducing liver damage and attenuating liver dysfunction [20].

Furthermore, albumin levels increase slightly in the group of rats consuming the fatty food and 10%Vv (27g/l) unlike the positive control rat group where there is a slight decrease (22g/l) at the end of treatment (Table 2). This result is similar to a study incorporating 20% mushrooms into the diet of obese rats, with respective albumin levels of 34 g/L and 25 g/L in the test rats and positive control rats [21]. Indeed, a high-fat diet can lead to a decrease in serum albumin levels in rats, although the effect varies depending on the rat strain and the specific composition of the diet [22, 23]. The small increase in albumin levels observed with the aforementioned mushrooms could be explained by the nutritional value of the mushroom, which, once incorporated into food products, increases the nutritional value, particularly protein [24]. This allows to normalize albumin levels in the animal's body during experiments. Normalization of albumin levels relies primarily on liver metabolism in response to nutritional intake, utilizing the binding protein FcRn. The FcRn receptor plays an important role in preventing excessive degradation [25].

5. Conclusion

Consumption of 10% *Volvariella volvacea* mushroom by rats fed a high-fat diet showed a beneficial effect by reducing leukocyte, alanine aminotransferase, and albumin levels compared to the control group of positive rats. This could be attributed to the various bioactive compounds present in this mushroom. Further research is needed to evaluate the effect of mushroom *Volvariella volvacea* on certain leukocyte parameters (including monocytes) in humans using an immunostimulation assay. The results of this research suggest that this mushroom, as a functional food containing bioactive compounds, could be used to prevent obesity and related diseases, thereby improving human health.

Abbreviations

Alb	Albumin
ALT	Alanine Aminotransferase
DW	Distilled Water
FIP	Fungal Immunomodulatory Protein

HFD	High Fat Diet
ILC2 cells	Group 2 Innate Lymphoid Cell
NK cell	Natural Killer Cells
Vv	*Volvariella volvacea*
WBCs	White Blood Cell
WHO	World Health Organization

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Author Contributions

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Conflicts of Interest

The authors declare no conflicts of interest.

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