



Research Article

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***In vivo* investigation of bone marrow stimulating activity of *Agaricus bisporus* (white button mushroom)**

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ABSTRACT

Investigation of in vivo bone marrow stimulating effect of Agaricuss bisporus (White bottom Mushroom, WBM). Water extract of white button mushroom was prepared using soxlet apparatus. Myelosuppression in wistar rats induced by busulfan induce bone marrow suppression model. Water extract of white button mushroom 250 mg/kg, p.o. was given to wistar rats for 21 days. At the end of treatment Hb, RBC, total WBC, neutrophil, basophile, eosinophil, lymphocyte, monocyte where measured using automated blood cells counter. Toxic dose of busulfan induced sever bone marrow depression in disease control and treatment control groups. There were significant increase in hemoglobin, red blood cell, differential white blood cell (WBC) and total platelet count in rats treated with water extract of WBM compared to disease control group rats. Beta glucan, antioxidant, protocatechuic acid and pyrocatechol present in water extract of WBM may be responsible for immunostimulating activity in busulfan induced bone marrow suppression model in wistar rats.

Keywords: Bone marrow suppression, white button mushroom, busulfan, whole blood cell count.

INTRODUCTION

Bone marrow suppression or myelotoxicity (adjective myelotoxic) or myelosuppression is the decrease in production of cells responsible for providing immunity (leukocytes), carrying oxygen (erythrocytes), and/or those responsible for normal blood clotting (thrombocytes).[1] Bone marrow suppression is a serious side effect of chemotherapy and certain drugs affecting the immune system such as azathioprine[2] .

Agaricuss bisporus, more commonly known as the white button mushroom (Edible Mushroom), is one of the oldest and popular fungi in the western diet. It is a regularly added to salads, stir fries, pastas, sauces, soups, pies and breakfasts. It is cultivated around the world for culinary purposes. In addition to its own unique flavor, eating this mushroom may provide important health and nutrition benefits when made a regular part of the diet [3].

The use of mushrooms extract and their bioactive compound associated antioxidants is becoming increasingly popular and could bring diverse physiological benefits to the consumer, such as protection against human diseases associated with oxidative stress, like coronary heart disease, oxidation associate pathologies, diabetes, infections (fungi, bacteria), immune system disorder and cancer. Recently, considerable attention is focused on anticarcinogenic bioactive compounds particularly those derived from medicinal or edible mushrooms [4].

In addition to the nutritional benefits of this mushroom, it may have useful medicinal properties that support health and wellbeing. In a study published in "BMC Complementary and Alternative Medicine" in 2011, researchers studied the effects of *Agaricus bisporus* on human immune cells in vitro. Researchers concluded the medicinal value of *Agaricus bisporus* was likely due to the carbohydrate-based chemicals called mannogalactans [5].

Hence, the major objective of the present study was to evaluate bone marrow stimulating effect of white button mushroom on bone marrow suppression.

EXPERIMENTAL SECTION

Agaricus bisporus (Edible mushroom) was collected from local market and authenticated by Dr. Parth Bhatt, Microbiologist, Associate professor at School of Science, RK University, Rajkot, Gujarat. Water extract of white button mushroom were prepared using Soxhlet apparatus. The water extract of WBM was concentrated to yield 8 % W/W.

Wistar albino rats of weighing 160-200 gm were used for the present study. The animals were procured from animal house, Department of Pharmacology, School of Pharmacy, RK University, Rajkot. All animals were housed at ambient temperature ($22\pm 1^{\circ}\text{C}$), in relative humidity ($55\pm 5\%$) and 12h/12h light dark cycle. Animals had free access to standard pellet diet and water given *ad libitum*. The protocol of the experiment was approved by the institutional animal ethical committee as per the guidance of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India.

Busulfan at a final concentration of 10 mg/ml in Na CMC was prepared and was infused doses of 25 mg busulfan/kg body weight each at daily for 21 days of treatment period which produced myelosuppression in rat. Busulfan is an alkylating agent with myeloablative properties and activity against non-dividing marrow cells and, possibly, non-dividing malignant cells [6-7].

The experimental animals were divided into three groups, six animals in each group.

Group I: Normal healthy control

Group II: Diseases control, busulfan induced myelosuppression rats (25 mg busulfan/kg)

Group III: Busulfan induced pancytopenia rats treated with white button mushroom (250mg /kg, p.o., 21 day)

All the studies were carried for a period of 21 days. After 21 days of treatment period blood samples were collected under fasting conditions and were subjected to estimations. Blood samples were collected in clean dry centrifuge tubes by retro orbital plexuses under light ether anesthesia and were collected in EDTA tube to prevent clot formation at room temperature.

Various hematological parameters like Hemoglobin content, Total RBC, Total WBC count, Differential WBC count, Neutrophil, Lymphocyte, Eosinophil, Basophil counts and Total lymphocyte count and Platelet count were estimated using fully automated hematology analyzer - Model XS-800i – Sysmex.

Results are presented as mean \pm SEM. Statistical differences between the means of the various groups were evaluated using one-way analysis of variance (ANOVA) followed by Tukey's test. Data were considered statistically significant at $P \leq 0.05$ and highly significant at $P \leq 0.001$. Statistical analysis was performed using INSTAT statistical software.

RESULTS AND DISCUSSION

Busulfan solution at concentration of 10 mg/ml in polyethylene glycol was prepared and infused in wistar rats at doses of 25 mg busulfan/kg body weight produced pancytopenia with significant reduction in hemoglobin, red blood cells, platelet and all white blood cell counts. Treatment with water extract of *Agaricus bisporus* (250 mg/kg, p.o, per day) results in significant decreased in Hb, RBCs, Platelet and differential WBC counts compared to diseases control group (Table 1 and 2).

Table 1: Effect of the white button mushroom on the hemoglobin level

Hematological Parameter	Normal control group	Disease control group	Treatment group
Hemoglobin	13.48 \pm 0.02	8.1 \pm 0.16*	12.28 \pm 0.18 [#]
RBCs	7.73 \pm 0.10	5.85 \pm 0.06*	7.6 \pm 0.12 [#]
Platelets	801.33 \pm 2.93	426.5 \pm 2.12*	788.67 \pm 1.26 [#]

Table 2: Beneficial effect of the white button mushroom on differential WBC count

Hematological Parameter	Normal control group	Disease control group	Treatment group
Neutrophil	2.98 +0.10	1.63 +0.12*	1.63 +0.08 [#]
Eosinophil	0.12 + 0.01	0.05 + 0.01*	0.11 + 0.01 [#]
Basophil	0.14 + 0.01	0.045 + 0.01*	0.13 + 0.01 [#]
Lymphocyte	1.78 +0.11	1.01 + 0.07*	1.48 + 0.09 [#]
Monocyte	0.43 +0.01	0.12 + 0.01*	0.36 +0.01 [#]

Values are expressed as Mean \pm SEM

*- Significant variation from normal control group ($p < 0.001$)

#- Significant variation from diseases control group ($p < 0.001$)

Normal control group: Receive vehicle (21 day, p.o.)

Disease control group: Treated with Busulfan

Treatment group: Treated with Busulfan + water extract of *Agaricus bisporus* (250 mg/kg, p.o, per day)

In treatment control group rats have significantly increased in RBCs and Hemoglobin level compared to disease control rats and non-significant difference between treatment and normal control group. Thus data indicate white button mushroom can be used in the anemic condition, bone marrow depression and cancer chemotherapy.

Busulfan induced bone marrow depression disease control rats exhibited significant decreased platelet count as compared to normal control group rats. While white button mushroom at 250mg/kg dose group in bone marrow depression rats significantly increased in platelet count as compared to disease control group. So, these data indicate that the white button mushroom have beneficial effect in thrombocytopenia, in bleeding disorder, dengue, gram negative sepsis and bone marrow depression disorder.

The monocyte, lymphocyte and neutrophil are the antigen presenting cell. They are protect the body from bacterial, viral, parasitic and other infection, and also benefited in immunodeficiency disorders like AIDS, cancer of immune system such as leukemia, lymphocytosis and other immunodeficiency disorders. Monocyte, lymphocytes and Neutrophil count significantly increased in the white button mushroom (250mg/kg) treated rats compared to disease control rats and no significant difference from normal control group. Thus it indicate that the white button mushroom help to preventing the body against various disease and infection.

Thus, we conclude the regular intake of white button mushroom can be useful in management of bone marrow suppression diseases and can possibly replace the current regime of expensive and various side of effect of colony stimulating factors, epoetin and other immuno stimulating agent. White button mushroom also replaced the anti-cancer agent which have bone marrow depression side effect. As well as detailed study in clinical trials of the bone marrow stimulant, may provide a new chemical entity for better management of bone marrow depression disease like immunodeficiency disorder and beneficial in cancer chemotherapy.

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