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Immunomodulatory Effects of Oyster Mushroom (*Pleurotus sajor-caju*) Extract In Balb/c Mice.

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Research Article

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ABSTRACT

Pleurotus sajor-caju (oyster mushroom, PSC) is widely used in culinary due to its various benefits on human health. However, no study has been reported on its immunomodulating properties. Cultivated mushroom powder was used for hot aqueous extraction using Soxhlet technique. The PSC extract was suspended into CMC and prepared into 3 groups: 0.2 g/kg, 1g/kg and 2g/kg. Balb/c mice were orally fed daily for 21 consecutive days. Treatment of mice with various concentrations of PSC showed increased populations of $CD3^+/CD4^+$, $CD3^+/CD8^+$, $CD14^+$ and $CD\ 19^+$ cells. Interestingly, PSC-treated mice demonstrated significant increase in $CD3^+/CD4^+$ and $CD19^+$ cells. Most importantly, mice treated with PSC showed improved $CD4^+/CD8^+$ ratio; which indicated potential immunostimulating activity by PSC. In addition, no physical changes and signs of mortality could be detected from PSC-treated mice. The results suggest that PSC may improve general immune status when consumed consistently as a daily food option.

INTRODUCTION

Immunomodulation and its prospect towards human's health exist since ancient times. Their exact roles in efficacy were scarcely understood despite widely practiced. Compounds such as proteins, peptides, and polysaccharides are commonly identified in immunomodulators and their response towards the immune system is essential either in up-regulating or down-regulating specific aspects of the host response [1]. Basically, these immunomodulators enhance body's natural resistance against infection [2]. The activity of immunomodulator is not only to boost immune cells but also to normalize them when the immune system is highly activated. Thus, it serves as an agent to maintain and regulate the existing balance of system [1,3]. These activities are executed mainly via phagocytosis stimulation, lymphoid cells stimulation, and cellular immune function enhancement [4]. Therefore, plants and herbs as natural immunomodulators have been gaining great priority in keeping health in check as well as to improve the immune system. Recently, many advances have been made towards understanding common culinary plants used in daily dietary and their high claimed medicinal values towards various diseases and ailments [5].

Mushrooms have a long history as a food source traditionally and also scientifically acclaimed for its medicinal properties. Mushrooms are considered healthy foods as they are low in calories, fats, essential fatty acids, and high in vegetable proteins, vitamin and minerals [6,7,8,9]. In addition, mushrooms possessed various medicinal benefits i.e. anti-bacterial, anti-viral [10]. anti-cancer [11]. reduce cholesterol level [12] and also immunomodulating effects [13, 14].

Grey oyster mushroom or scientifically known as *Pleurotus sajor caju* (PSC) is an edible mushroom commonly found in tropical regions. This edible mushroom is presumed to possess considerable

importance in the human diet as they are rich in non-starchy carbohydrates, dietary fibre, β-glucans, minerals and vitamin-B and is low in fat content.[15] Due to these nutritional values, PSC has been commercially grown to meet high demands especially from food and nutraceutical industries.[16] Apart of the nutritional values, this PSC also possessed various significant medicinal properties . Recent studies showed that PSC possessed antioxidant and anti-cancer properties [17] and has a potential in modulating the immune system.[18] However, to date no such studies have been conducted on the immunomodulatory properties of this mushroom in *in vitro* animal models. The present study investigated the immunomodulatory effect of hot aqueous extract of grey oyster mushroom or PSC, a mushroom commonly available in Malaysia by assessing their effects on lymphocyte subpopulations and cytokines level in Balb/c mice.

MATERIALS AND METHODS

Chemicals

In the present study, the following reagents were used: RPMI 1650 medium was purchased from Sigma chemical Co. (USA); carboxyl methyl celulose (CMC) was purchased from Sigma chemical Co. (USA); phosphate buffer saline, PBS (Amresco, USA); surface marker antibodies PerCP CD3, FITC CD4, PE CD8, FITC CD14 and FITC CD 19 were purchased from BD Pharmingen™(USA).

Preparation of PSC extract

Cultivated dried mushrooms were obtained from National Kenaf and Tobacco Board of Malaysia. The mushrooms were ground and the powder was extracted with hot water using percolation and Soxhlet techniques. The extracted solution was then freeze-dried into lyophilized powder form. The freeze-dried extract was then suspended into CMC (0.5% viscosity) and prepared into 3 different concentrations i.e. 0.2 g/kg, 1g/kg and 2g/kg.

Animals and experiment design

Male Balb/c mice (age 6-8 weeks; 20-25g weight) were use in this experiment. The mice were housed in polystyrene cages in an air-controlled room. All animals were maintained at animal house and tap water was given *ad libitum*. The animals were acclimatized for 1 week prior to experiment. The mice were divided into 5 groups i.e. Untreated (control group); CMC solution at 0.3 ml/mice daily dose (vehicle), 0.2g/kg PSC, 1g/kg PSC and 2g/kg PSC. Animals were orally fed using gavage daily for 21 days. Pre- and post-treatment serum was collected from all mice groups during the experiment duration.

Organ and body weight

Both pre- and post-treatment body weights of animals were recorded. At the end of the study, the mice were sacrificed by cervical dislocation. The spleens were removed, weighed, and placed in sterile 15ml centrifuge tubes of 1 ml RPMI-1640 medium then stored at 4°C for further analyses.

Preparation of single cell suspension

Each spleen was placed on a sterile culture plates and crushed by compressing between two sterile glass slides. Then the homogenized suspension was collected from the tissue plates and filtered though cell strainer 70 µm (*BD* Falcon™) into a new sterile centrifuge tube. The tubes were centrifuged at 1500 rpm for 5 min at 10°C. The supernatant was discarded, and the pellet was resuspended with 1 ml of PBS. Cell counting was performed using hematocytometer and a total of 5×10^5 cell was prepared and aliquoted into 100µl of RPMI in a flow cytometry round-bottom tubes.

Splenic T lymphocyte subsets assay by flow cytometry

Splenocytes were stained by respective surface marker antibodies namely PerCP $CD3^+$, FITC $CD4^+$, PE $CD8^+$, FITC $CD14^+$ and FITC $CD19^+$. After staining process, samples were incubated in the dark area for 20 minutes at 4°C. Later, the samples were suspended with 1ml PBS, mixed and centrifuged again. After carefully aspirating supernatant out, the stained cells were resuspended with 500 µl RPMI and the cells were stored at 4°C, overnight in the dark. Samples were then analyzed using FACSCanto™ flow cytometry to identify lymphocyte cell subsets distribution.

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Statistical analysis

Data are represented as means ± SD of each mouse from different groups. The experiments were carried out in triplicates. Statistical analysis was carried out using Wilcoxon W test and Mann Whitney U test by statistical program for social science (SPSS) version 20.0. Differences were considered as statistically significant when the *p* values were below 0.05 ($p<0.05$).

RESULTS

Effects of PSC extract on body weight

Daily oral administration of PSC extract for 21 days did not result in any mortality. No signs of toxicity were observed as demonstrated by changes in the terminal body weights as well as the body weights monitored pre- and post-treatments (Figure 1). Analysis on mice pre-and post treatment body weights shows PSC has no significant differences, thus exhibits less influence on the body weight.

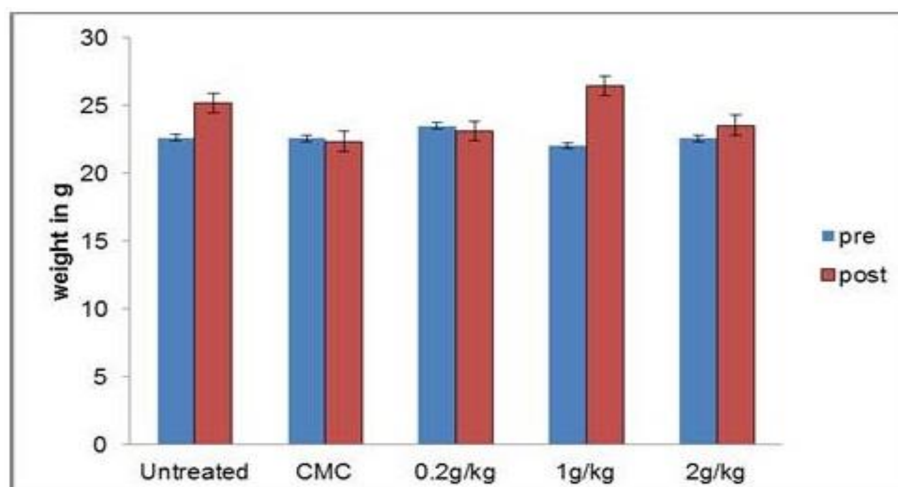
Effects of PSC extract on cell subsets distribution

The mice administered with PSC extract showed increased lymphocyte subsets percentage ($CD3^+/CD4^+$, $CD3^+/CD8^+$, $CD19^+$ and $CD14^+$) after administration with various concentrations of PSC extract (Figure 2 a-d). The results demonstrated significant increased of $CD3^+/CD4^+$ and $CD19^+$ cell subsets when comparison made to untreated group. The PSC-treated mice also showed increased $CD3^+/CD8^+$ and $CD14^+$ even though the percentage of increment was not significant. Interestingly, populations of $CD3^+/CD4^+$, $CD3^+/CD8^+$, $CD19^+$ and $CD14^+$ in treated mice showed the highest subset populations when administered with 0.2g/kg of PSC extract. Most importantly, the PSC-treated groups showed significant increased in the ratio of $CD4^+/CD8^+$ cells (Table 1).

Table 1: Effects of PSC on the percentage ratio of splenic T lymphocyte subpopulations. Values represented the mean ± SD, which were analyzed by Wilcoxon W test and Mann Whitney U test to evaluate the difference between PSC groups and untreated group.

Group	% CD4	% CD8	CD4/CD8 ratio
Control	4.82 ± 0.72	8.60 ± 0.87	0.56
CMC	3.13 ± 0.55	9.88 ± 1.14	0.32
0.2g/kg	18.00 ± 2.83	11.90 ± 1.87	1.52
1g/kg	15.85 ± 2.22	8.88 ± 1.53	1.78
2g/kg	9.33 ± 0.51	8.14 ± 0.65	1.15

Figure 1: Effect of PSC extract on mice pre- and post-body weight after orally administered with PSC extract for consecutive 21 days. Values represented the mean ± SD, which were analyzed by Wilcoxon W test and Mann Whitney U test to evaluate the difference between PSC groups and untreated group. *$p< 0.05$ versus control.



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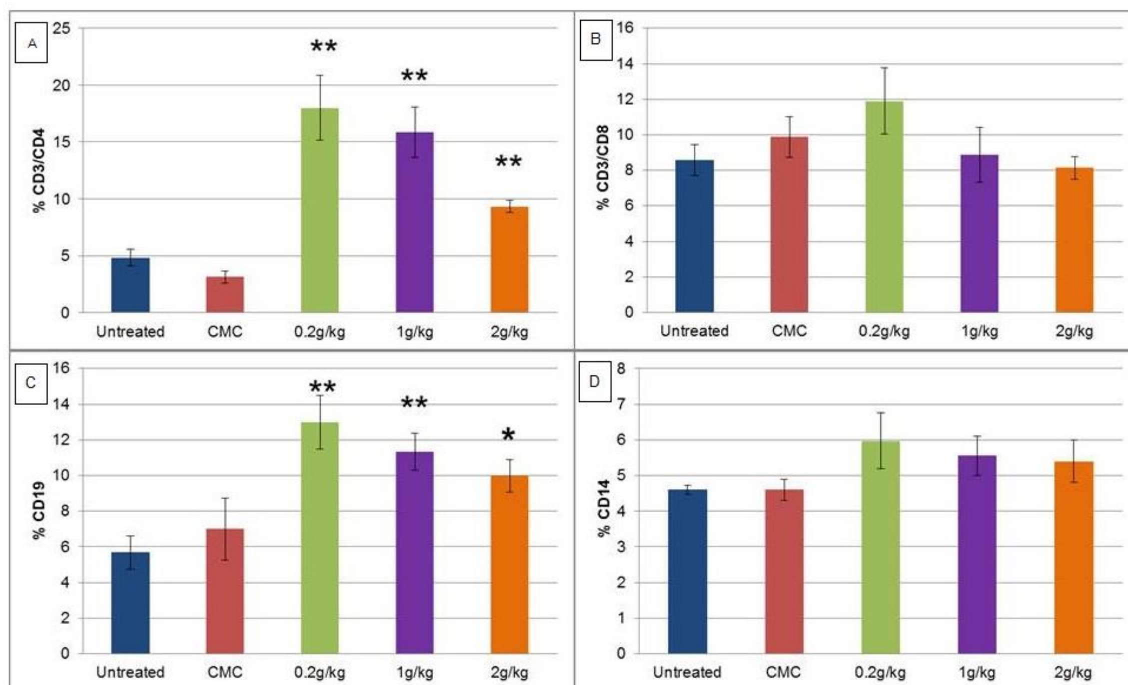

Figure 2: FACS analysis of (a) CD3+ CD4+ helper T cell (b) CD3+ CD8+ cytotoxic T cells (c) CD19+ B cells and (d) CD14+ macrophages/monocytes percentages on lymphocyte cells of splenocyte populations in all mice group after 21 days oral administration with PSC extract. Values represented the mean ± SD, which were analyzed by Wilcoxon W test and Mann Whitney U test to evaluate the difference between PSC groups and untreated group. *$p< 0.05$ versus control. **$p< 0.01$ versus control.

DISCUSSION

Edible mushrooms which are usually used in culinary or alternative medicine may have important effects on health or even in treating diseases. Previous studies showed that many species of edible mushrooms have exhibited immunomodulatory activities [19]. This may be due to the presence of numerous fungal compounds such as polysaccharides (mostly β- and some α-glucans), proteoglycans, and proteins which have been extensively reported for their immunomodulatory potency and activity [1]. Recently, much attention has been paid to dietary components for multiple physiological activities. Grey oyster mushroom or PSC is widely cultivated and consumed in Malaysia due to its flavorful taste and nutritional properties [16]. Previous studies showed significant multiple effects of PSC to treat various diseases [11,13,14,10]. However, to date, no specific studies reported on the immunological effect of PSC. The importance of disclosing its immunological properties will initiate various new applications, including value-added foods or nutritional supplements.

In the present work, we studied the effect of oral administration of aqueous extract of PSC in an in vivo model. Oral administration of PSC showed that Balb/c mice treated with PSC extract showed significant increase of certain lymphocyte subpopulations. The importance of the percentage and ratio of T helper cells ($CD4^+$) and cytotoxic T cells ($CD8^+$) populations have become important in determining the balance state of immunomodulation and response to the homeostasis of intrinsic immune system [20]. Previous studies have shown that some food sources for examples peptides from bovine, soybean and whey proteins possess immune-stimulating capacity by increasing the percentage of $CD4^+$ cells or $CD4^+/CD8^+$ ratio [21, 22, 23]. Flow cytometric analysis of splenocytes with $CD3^+/CD4^+$ antibodies in this study showed significant increase of T helper cells population in all treated groups compared to the control groups (Figure 2a). However, no significant increase in $CD3^+/CD8^+$ cells population could be observed from PSC-treated mice; even though mice treated with 0.2g/kg and 1g/kg of PSC extract demonstrated higher cytotoxic T cells population when compared to the control groups (Figure 2b). The finding corresponded with Rubel *et al.* where the animals treated with *Ganoderma lucidum* reported a selective decrease in the population of $CD3^+$ and $CD8^+$ in spleen cells [24]. Most importantly, this study demonstrated significant increased $CD4^+/CD8^+$ cells in all PSC-treated groups.

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The fungal cell wall of mushroom is known to comprise chitin, β-glucans and mannan-containing glycoproteins [25] β-glucan, a common polysaccharide in mushrooms have been described as a potent immunomodulator with specific activity for T cells and antigen-presenting cells such as monocytes and macrophages [1]. Macrophages, dendritic cells (DCs) and B lymphocytes are the principal cell types involved in the presentation of exogenous antigens to CD4+ T lymphocytes. All these cells express receptors i.e. surface immunoglobulins (sIg) in B cells [26], and Fc receptors (FcRs) [27] as well as different mannose receptors (MRs) in macrophages and dendritic cells [28, 29] that mediate the uptake of antigens [30] allowing presentation of the antigens. The results obtained in this study demonstrated PSC-treated mice produced significantly higher B cells population. However, $CD14^+$ cells which predominantly expressed by macrophages and also DCs found to be expressed slightly higher in PSC-treated mice. Previous studies showed that β-glucan also stimulated the proliferation of monocytes and macrophages and have potent hematopoietic activities [31,32]. Shibata *et al*. reported that β-glucan involved in alteration of macrophage morphology and increased the phosphatase activity and lipopolysaccharide (LPS) stimulated NO production [33]. Recent evidences also suggest that mushrooms or the β-glucan modulates the production of pro-inflammatory cytokines [24,34,35].

In conclusion, the present study has demonstrated that PSC has the potential to non-specifically enhanced the humoral and cellular components of the host immune systems. Moreover, the positive immunomodulation by PSC is most likely attributed to the stimulation of helper T cells; as indicated by increasing ratio of $CD4^+/CD8^+$ cells. Also, there is notable pattern of declining percentage rate of lymphocytes subpopulations as the treatment dosage increases which may explain optimum dosage and its efficacy. Therefore, PSC may potentially improve general immune function when consumed in optimal portion and regularly as a daily food options.

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