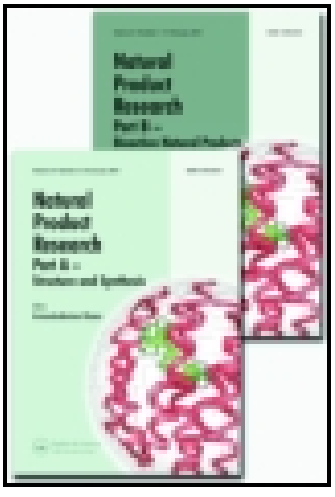

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SHORT COMMUNICATION

Haematopoiesis radioprotection in Balb/c mice by an aqueous mycelium extract from the Basidiomycete *Pleurotus ostreatus* mushroom

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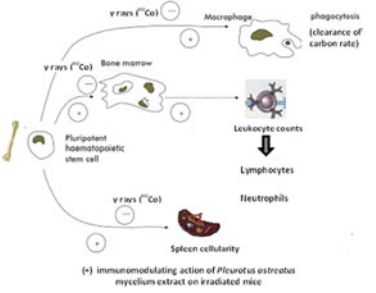

The study examined the radioprotective activity of an aqueous extract from *Pleurotus ostreatus* mycelium administered to Balb/c mice. Male mice were whole-body irradiated on day 0 (^{60}Co, at 0.43 Gy/min) and divided into two groups. The extract was administered intraperitoneally to one group (100 mg/kg) on days −10 to −6 and −2 to +1 with respect to the irradiation. The irradiated-control group was injected with saline solution; non-irradiated mice were used as negative controls. The radioprotective effect was evident by increases in bone marrow cellularity (5.1×10^6/femur vs. 1.1×10^6/femur in saline-control mice, $p < 0.05$), leucocyte counts (10.5×10^9/L vs. 4.5×10^9/L, $p < 0.05$), and spleen cellularity (11.2×10^7/spleen vs. 6.2×10^7/spleen, $p < 0.05$). The extract stimulated macrophage phagocytic activity as judged by a faster rate of carbon clearance in terms of absorbance ratios (1.62 vs. 2.01, $p < 0.05$). Therefore, this extract may be a candidate therapeutic agent with radioprotective activity for haematopoiesis damage, particularly to cells involved in immune function.

Keywords: haematopoiesis; immunomodulation; mushroom; *Pleurotus*; radioprotection

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1. Introduction

Radiotherapy in cancer treatment commonly results in a depression of the immune system, especially as a result of destruction of lymphoid and bone marrow cells. Therefore, the development of a radioprotective agent is expected to be an essential part of cancer therapy (Zhuang 2009). *Pleurotus* spp. (Pleurotaceae) is a popular cultivated edible mushroom with medicinal properties. Crude extracts and biomolecules isolated from both fruiting bodies and mycelia of *Pleurotus* spp. have been documented to possess antioxidant, antitumour and immunomodulating effects (Khan & Tania 2012). As mushroom cultivation takes several months to complete fruiting body development during solid-state fermentation, an alternative and promising approach is to search new safe and healthy products from mushroom mycelia-submerged cultures (Chang & Wasser 2012). In a previous study, we reported the immunomodulating activity of a hot-water extract from *P. ostreatus* mycelium against the immunosuppression caused by cyclophosphamide in mice (Morris et al. 2003). The presence of polysaccharides and large quantities of phenols, flavonoids and alkaloids was confirmed in the extract (Morris et al. 2014). However, some other biological activities of *Pleurotus* mycelial extracts may yet still be identified. In this article, we assessed the radioprotective effects of an extract from *P. ostreatus* mycelium administered in a prophylactic schedule to Balb/c mice that subsequently underwent whole-body irradiation.

2. Results and discussion

2.1. *Haematological analysis*

With the exception of bone marrow cells, the rest of the investigated parameters in mice treated with *Pleurotus* extract was restored to the levels found in non-irradiated (negative control) group. The recovery of bone marrow cellularity, leucocyte counts and spleen cellularity at the time point measured was higher than in animals treated with saline (Figure 1). Compared with levels in mice that did not receive the extract, bone marrow cells increased 4.6-fold (5.1×10^6/femur vs. 1.1×10^6/femur in saline-control mice, $p < 0.05$). Overall levels of white blood cells

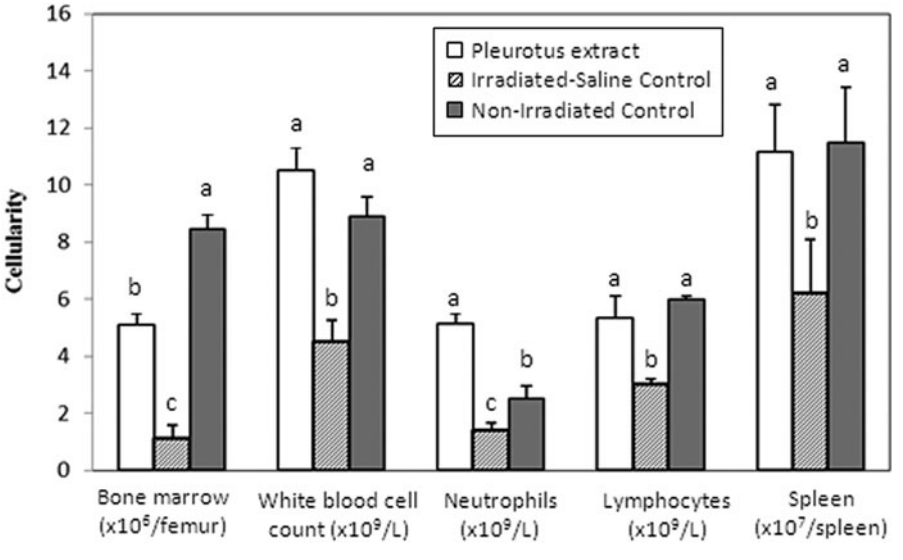

Figure 1. Radioprotective effects of *Pleurotus* hot-water extract on haematopoiesis in irradiated Balb/c mice. Values shown are the mean (±SE) of each group ($n = 10$). Different letters indicate significant differences among groups (Kruskal–Wallis, Student–Newman–Keuls, $p < 0.05$).

in circulation were also significantly higher among the extract-treated mice, with levels 2.3-fold higher than in the vehicle-treated counterparts ($p < 0.05$). In the blood, the pool of neutrophils was also increased up to 3.7-fold relative to the values in the control mice (5.1×10^9/L vs. 1.4×10^9/L, $p < 0.05$). Similarly, the lymphocyte pool was also significantly higher (1.8-fold) due to the extract administration (5.35×10^9/L vs. 3.01×10^9/L, $p < 0.05$). Last, it was also clear that treatment with the mushroom-derived materials led to increases in splenic cellularity relative to levels seen in mice that received only saline (11.2×10^7/spleen vs. 6.2×10^7/spleen), a change of 1.8-fold ($p < 0.05$). It is plausible that in mice administered with *Pleurotus* extract, bone marrow-derived cells would migrate at a faster rate to other lymphoid organs such as the thymus where a moderate hyperplasia was found in the histological analyses (data not shown) and the spleen (Figure 1). Further studies are needed to distinguish intact normal cells with an effective function improved by administration of mycelium extract from other cells. The amounts of estimated cells might be the sum of those pre-existing protected cells (i.e. against oxidative damage induced by ionising radiation) with an increased survival, and in addition, the generation of new cell progenies stimulated by components of *Pleurotus* extract. Not only immune effector functions, but also antioxidant and DNA repair mechanism could be enhanced by mycelium extract.

The mechanism of the haematopoiesis activation exerted by *Pleurotus* extract is unclear. The increased neutrophil and lymphocyte counts were believed to be related to a stimulation of haematopoietic cytokines by molecules present in mushroom material such as polysaccharides. The induction of a marked increase in the amounts of colony-stimulating factors and IL-3 by polysaccharides results in maturation, differentiation and proliferation of the immunocompetent cells for host defence mechanisms (Wasser 2002). Purified polysaccharides from *Ganoderma lucidum* mycelium can induce the proliferation of human peripheral blood mononuclear cells. Effects on innate immunity include the activation of Toll-like receptor (TLR-4), a key receptor for innate immune response, expressed by murine macrophages and human dendritic cells, as well as murine B cells (Chan et al. 2007).

2.2. *Peritoneal exudate cells and carbon clearance test*

The treatment with the *Pleurotus* extract significantly increased the number of cells (primarily macrophages) in the peritoneal cavity of mice compared with levels seen in irradiated-saline control hosts (Table 1). In the experimental group, peritoneal exudate cells (PEC) number was restored to levels found in non-irradiated mice. In the study to evaluate the effects of the extract on *in vivo* phagocytic activity by measuring carbon clearance in peripheral blood (as an index of the phagocytic activity of liver and spleen), a low ratio was deemed to correspond to a high clearance of carbon from the blood. The data show that the treatment with the extract potentiated

Table 1. Effects of *P. ostreatus* hot-water extract on the number of PEC and macrophage phagocytic activity of irradiated Balb/c mice.

	Pleurotus extract	Irradiated-saline control	Non-irradiated control
Number of PEC ($\times 10^6$/mouse)	4.61 ± 1.43a	1.82 ± 0.65b	3.41 ± 0.57a
Macrophage phagocytic activity (absorbance ratio at 5 min)	1.62 ± 0.12b	2.01 ± 0.31a	–

Notes: Values are means ± SE, $n = 10$. Different letters indicate significant differences, $p < 0.05$. Kruskal–Wallis followed by Student–Newman–Keuls for the number of PEC and the Student's *t*-test for phagocytic activity. (–) The value was used in the estimation of absorbance ratios.

the activity of the host monocyte–macrophage system (relative to that in the irradiated saline-treated mice) (Table 1).

These results were in keeping with the finding of another study wherein water-soluble fractions of *P. ostreatus* mycelium exerted modulating effects on macrophage activation *in vitro* as reflected in enhanced glucose consumption and acid phosphatase activity by the treated cells (Morris et al. 2007). The noted increases in macrophage activation in that and the present study might be related to binding of one or more extract components to receptors found on macrophage surface such as glucan receptors (i.e. dectin-1). Polysaccharides appear to be the most important component with respect to antitumour effect and on the average, 1.5% of mycelium extract dried mass consists of β-1,3-1,6-glucans (Morris et al. 2014). Anyhow, the use of a mixture of compounds may be useful since different molecules would modulate distinct intracellular signalling and produce synergistic effects *in vivo*.

These findings are in agreement with those of a clinical trial wherein patients with different types of cancer (hepatic, lung, gastric, colorectal and nasopharyngeal) who were undergoing chemotherapy or radiotherapy received a nutritional supplement containing polysaccharides extracted from six different mushrooms. Those patients showed an increase in white blood cell counts, activation of their monocyte–macrophage system and alleviation of toxic reactions caused by anticancer therapies (Novaes & Fortes 2005).

3. Conclusion

The hot-water extract obtained from *P. ostreatus* mycelium by submerged fermentation, in light of the effects on haematopoiesis in hosts that would otherwise remain devastated by ionising radiation could be considered as a good candidate for radioprotective agent, particularly to cells involved in immune function. These results suggest the use of this extract as a potential agent for clinically accelerating the haematological recovery of patients under radiotherapy, at least for prophylactic purposes. The isolation of the active components should enable us to more precisely dissect and distinguish the physiological mechanisms regulating this radioprotective activity.

Supplementary material

Experimental details relating to this article are available online.

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Disclosure statement

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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