Zymosan exerts radiation protection on AHH-1 and HIEC cells through TLR2/4-MyD88-G-CSF/GM-CSF/IL-12/IL-6 pathway

Yue-zhi Zhang  
Binzhou Medical University

Shu-jing Ge  
970 Hospital of Chinese PLA

Qing-zhen Leng  
970 Hospital of Chinese PLA

Jian-jun Ma  
970 Hospital of Chinese PLA

Hanchen Liu (✉ fnd789@163.com)  
970 Hospital of Chinese PLA  https://orcid.org/0000-0002-3647-2072

Research article

Keywords: Zymosan, radiation protection, MyD88, cytotoxicity

DOI: https://doi.org/10.21203/rs.3.rs-37397/v1

License: © This work is licensed under a Creative Commons Attribution 4.0 International License.  
Read Full License
Abstract

**Background:** This study was to confirm the radiation protective effect of different doses of zymosan on AHH-1 and HIEC cells irradiated at different times and different doses, and further to explore whether zymosan exerts a radiation protection mechanism by targeting TLR2/4.

**Methods:** AHH-1 and HIEC cells were respectively administered to Zymosan at 0, 20, 40, 80 and 160 μg/ml. CCK-8 and cell flow cytometry were used to detect the cell activity and apoptosis at 24 h, 48 h, and 72 h after administration to determine the dose-limiting toxicities of zymosan. Twelve hours before irradiation, cells were treated with zymosan at 0, 5, 10, and 20 μg/ml, and then irradiated with 4Gy X-rays. The cell activity and apoptosis were measured by CCK-8 and cell flow cytometry at 24 h to determine the optimal dose of zymosan. LPS was used as a positive control to compare the protective effect of zymosan. The cells were treated with MyD88 inhibitors to explore the protective mechanism of zymosan.

**Results:** The activity of AHH-1 and HIEC cells treated with different concentration of zymosan at different time was not affected and the apoptosis of cells was not promoted. The radiation protection effect of Zymosan pretreated cells on cells is dose-dependent. After zymosan pre-treated the cells, its radiation protection effect on the cells was dose-dependent. The higher zymosan's concentration was, the stronger the activities of AHH-1 cells and HIEC cells were, and the lower the apoptosis rate was. The activity of cells pretreated with zymosan was higher than that pretreated with LPS at the same dose (20 μg/ml), and the cell apoptosis rate was lower than that pretreated with LPS. After zymosan pretreated AHH-1 and HIEC cells, TLR2/4-MyD88-G-CSF/GM-CSF/IL-12/IL-6 pathway was activated.

**Conclusion:** Zymosan is nontoxic to cells and has better radiation protection effect than LPS. Its mechanism of action is related to the activation of the TLR2/4-MyD88/G-CSF/GM-CSF/IL-12/IL-6 pathway.

Background

Nuclear energy is a kind of clean energy, which can effectively deal with the energy crisis. The use of nuclear energy is a "double-edged sword", and the development and utilization of nuclear energy is also accompanied by security risks and challenges. The accompanying ionizing radiation will produce a large number of high-energy particle flow, which is very harmful to the environment and human body [1]. Recently, X-ray, γ-ray and neutron current in nuclear radiation have been widely used in the fields of industrial and agricultural production, radiation medicine, nuclear technology research and national defense [2-4]. In the national defense security, especially in the frontier island defense missions, the application of new nuclear weapons such as nuclear submarines and some nuclear power equipment has great potential harm to the body of officers and soldiers and the number of soldiers and people exposed to radiation is increasing year by year [5]. Therefore, all kinds of radiation generated by nuclear reactions must be effectively protected to ensure the health of workers and the safe use of nuclear
energy. Based on the above reasons, it is of great practical significance to study the protection of nuclear radiation damage.

At present, the best anti-radiation drug approved by the FDA in the world is WR-2721, which is equipped by the US Army. However, due to its obvious side effects, the use of WR-2721 is restricted to a certain extent [6]. Most of the radiation prevention and treatment drugs currently in the research stage have shortcomings such as unclear effects, unclear mechanisms or large toxic reactions, and they are only limited to preventive administration. And when they were injected after radiation, there was no clear treatment effect. TLR (Toll-like receptors) is a class of evolutionarily conserved pattern recognition receptors (PRR) that participate in the innate immunity of the body [7]. Up to now, 11 kinds of TLR have been found in mammals, and they have common similarities in signal transduction pathway and biological activity [8]. Studies have shown that most signaling pathways of TLRs can rely on the myeloid differentiation factor 88 (MyD88) pathway to activate nuclear factor kappa beta (NF-κB), thereby inhibiting apoptosis and promoting cell proliferation. When NF-κB is activated, on the one hand, NF-κB is involved in cell proliferation and differentiation, and inhibits cell apoptosis; on the other hand, NF-κB can activate the transcription and expression of multiple downstream target genes, such as interleukin (IL)-3, tumor necrosis factor (TNF)-α, granulocyte colony-stimulating factor (G-CSF), recombinant human macrophage colony stimulating factor (M-CSF), granulocyte-macrophage colony stimulating factor (GM-CSF), etc [9]. Existing studies have found that the radiation protection effect of activating TLR2 and TLR4 is significantly better than that of other TLR ligands and signaling pathways. The radiosensitivity of TLR2/TLR4 knockout mice was significantly increased, and the administration of TLR2/TLR4 agonists can effectively improve the survival rate of mice and reduce the damage of bone marrow, small intestine and other radiosensitive tissues [10, 11]. However, although the traditional TLR2 agonists Pam2 and Pam3, and TLR4 agonist lipopolysaccharide (LPS) have been proven to have certain radiation protection effects, they are highly toxic, which seriously limits its military application.

Therefore, there is an urgent need to develop high-efficiency and low-toxicity prevention and treatment drugs aiming at new targets of ionizing radiation.

Most polysaccharides extracted from nature have significant immunomodulatory effects, and they have many advantages, such as wide source, low toxicity, no carcinogenic effect and high safety. Many studies found that some water-soluble polysaccharide could reduce the radiation injury of animals and enhance the rapid recovery of radiation injury [12, 13]. Zymosan, also called as yeast polysaccharide, is mainly prepared from the yeast fungus wall. It contains a type of dextran linked by β-1,3 glycoside bond. It is also the first dextran found to have immunological activity [14]. Zymosan shows a good protection effect in radiation protection, and the protection effect can reach 70% [15]. Zymosan has been shown to be a TLR2/4 agonist, which can activate both TLR2 and TLR4 signaling pathways [16]. However, the mechanism of radiation protection effect of zymosan is rarely explored, and it is still a research hotspot.

In this study, we confirmed the toxic and side effects of zymosan, further clarified the effect of radiation protection at different times and different doses from the cellular level, and explored the mechanism of
radiation protection of zymosan targeting TLR2/4. It provided theoretical and experimental basis for the development of new, safe and effective radiation protection drugs in the future.

**Methods**

**Cell culture**

Human peripheral blood B lymphocyte (AHH-1, BNCC331188) was purchased from BNBIo.com (Beijing, China) and human intestinal epithelial (HIEC, MZ-0792) cells were purchased from Mingzhoubio.com (Zhejiang, China). The cells were cultured in DMEM medium containing 10% fetal bovine serum at 37 °C and 5% CO$_2$.

**Effect of zymosan on cell toxicity**

AHH-1 and HIEC cells were respectively administered to zymosan (tlrl-zyn, InvoGen) at 0, 20, 40, 80 and 160 μg/ml. CCK-8 and cell flow cytometry were used to detect the cell activity and apoptosis at 24 h, 48 h, and 72 h after administration to determine the dose-limiting toxicities of zymosan.

**Determination of the best dose of zymosan on cell radiation protection**

Twelve hours before irradiation, cells were treated with zymosan at 0, 5, 10, and 20 μg/ml, and then irradiated with 4Gy X-rays. CCK-8 and cell flow cytometry were utilized to detect cell activity and apoptosis at 24 h to determine the optimal dose of zymosan.

**Cell grouping**

Cells are randomly divided into 4 groups: normal control group (Control, cells are normally cultured), only irradiation group (Model, cells are irradiated with 4Gy radiation dose), positive control group (lipopolysaccharides, LPS, cells were treated with 20 μg/ml LPS 12 h before irradiation), the best dose group of (zymosan, cells were treated with 20μg/ml zymosan 12 h before irradiation.

**CCK-8**

Cells ($10^3$-$10^4$ cells/well) were cultured in an incubator containing 5% CO$_2$ at 37°C for 24 hours, then added with 10 μl CCK-8 solution (GipBio, Shanghai, China) and mixed well. Then were continued to be incubated for 4 h and oscillated in a microplate reader. The absorbance of each hole at 450 nm was measured by zero adjustment of blank control hole. The cell survival rate (%) =\([\text{As-Ab}]/(\text{Ac-Ab})\]×100.

As = Absorbance of experimental pores containing cells, medium, CCK-8 and compounds to be tested.

Ab = Absorbance of blank pores containing medium and CCK-8.

Ac = Absorbance of control pores containing cells, medium and CCK-8.
**Flow cytometry**

Annexin V-FITC/PI kit (CA1020, Solarbio, Beijing, China) was used to detect cell apoptosis. Cells cultured for 24 hours (1 × 10^6 cells/time) were collected and washed with precooled PBS. Cells were suspended in 1 mL of 1× binding buffer and centrifuged at 300 ×g for 10 mins. Then the cell concentration was adjusted to 1×10^6 cells/mL with 1 mL of 1× binding buffer. 100 µL of cells were added with 5 µL of annexin V-FITC and incubated in dark for 10 mins at room temperature, then added with 5 µL of propidium iodide (PI) and incubated for 5 mins. Finally, the volume of cell incubation suspension was adjusted to 500 µL by PBS and tested on the computer within one hour.

**A preliminary study on the protective mechanism of zymosan**

To determine whether zymosan exerts radiation protection through the TLR2/4-MyD88-G-CSF/GM-CSF/IL-12/IL-6 pathway, we used MyD88 inhibitors (10µM ST2825, MedChemExpress, Shanghai, China) to treat cells [17], and analyzed the expression of this pathway related proteins.

**Western blot**

The expression of TLR2, TLR4, MyD88, G-CSF, IL-12 and IL-6 in each cell line was detected by Western-blot method. 40 µg of samples were added with 10% SDS-PAGE buffer and electrophoresed using Bio-Rad electrophoresis device. Then they were transferred to the PVDF membrane for 30-60 mins, blocked, and incubated with the following primary antibody overnight: anti-rabbit TLR2 (1:1000, PA5-17492, ThermoFisher, Shanghai, China), TLR4 (1:200, 48-2300, ThermoFisher), MyD88 (2 µg/mL, PA5-19919, ThermoFisher), G-CSF (1:1000, PA5-86821, ThermoFisher), IL-12 (0.8µg/mL, PA5-18741, ThermoFisher), IL-6 (1:1000, P620, ThermoFisher) and β-actin (1:8000, PA1-16889, ThermoFisher). After rewarming, the membranes were incubated with secondary antibody Ig G (0.3µg/mL, A32731, ThermoFisher) at room temperature for 1 h. Then they were washed and colored with ECL luminescent substrate for 3-5 mins. Protein expression levels were scanned, quantified and finally normalized relative to β-actin using the Image J (NIH) software.

**Statistical analysis method**

Prism5.01 statistical analysis software was used for data processing, and the results were expressed as mean ± standard deviation (X±SD). The data analyses among multiple groups were assessed by one-way analysis of variance (ANOVA), and Tukey test was exerted for subsequent analysis. p<0.05 indicates that the difference is statistically significant.

**Results**

**Effect of zymosan on cell activity**

As demonstrated in Figure 1A, the activity of the cells was not inhibited when AHH-1 cells were treated with different concentrations of zymosan (20, 40, 80 and 160 µg/mL). The activity of the cells was not
inhibited at different processing time (24, 48 and 72 h). Similarly, the activity of the HIEC cells wasn’t also inhibited when they treated with different concentrations of zymosan at different processing time (Figure 1B). These results showed that zymosan did not inhibit cell activity.

Effect of zymosan on apoptosis

In order to further confirm that zymosan has no toxicity to cells, we further detected the apoptosis of AHH-1 and HIEC cells. Figure 2 demonstrated that the apoptotic rates of AHH-1 and HIEC cells treated with zymosan at different concentrations (20, 40, 80 and 160 µg/mL) for 24 hours were respectively less than 0.6% (Fig. 2A) and 1.1% (Fig. 2B). In figure 3, the apoptotic rates of AHH-1 and HIEC cells treated with zymosan for 48 h were less than 0.8% (Fig. 3A) and 0.7% (Fig. 3B), respectively. Furthermore, the apoptotic rates of AHH-1 and HIEC cells were separately less than 1.1% and 0.7% when they were treated with zymosan of different concentrations for 72 h (Fig 4). The above results indicated that zymosan did not promote apoptosis.

Radiation protection of zymosan on cells

The radiation protection effects of zymosan pretreatment on cells were as exhibited in the figure 5. The protective effect of zymosan on cells was dose-dependent. The higher the concentration of zymosan was, the stronger the activity of AHH-1 cells and HIEC cells was (Figure 5A, B) and the lower the apoptosis rate of AHH-1 cells and HIEC cells was (Fig. 5 C,D). This meant that zymosan pretreatment could effectively reduce the radiation damage to cells.

Comparison of the radiation protective effects of LPS and zymosan on cells

Further using LPS as a positive control to conduct the experiment, it was found that the same dose (20 µg/mL) of zymosan is superior to LPS in preventing radiation damage. As shown in Fig. 6A and B, the cell activity significantly increased after AHH-1 and HIEC cells were pretreated with LPS and zymosan, respectively, and the cell activity of zymosan pretreatment group was higher than that of LPS pretreatment group. And the detection of apoptosis of cells (Fig. 6 C, D) revealed that the apoptosis rates of AHH-1 and HIEC cells were significantly reduced after being pretreated with LPS and zymosan, and the apoptosis rate of cells pretreated by zymosan was lower than that of cells pretreated by LPS. This showed that zymosan had better radiation protection than LPS.

Preliminary study on radiation protection mechanism of zymosan

To further investigate whether Zymosan affects radiated cells through the TLR2/4-MyD88-G-CSF/GM-CSF/IL-12/IL-6 pathway, the expressions of pathway-related proteins were quantified by Western blot analysis. The results were shown in Figure 7 and 8. After AHH-1 and HIEC cells pretreated with 20 µg/mL zymosan, the TLR2/4-MyD88/G-CSF/GM-CSF/IL-12/IL-6 pathway was activated, and the expressions of the above proteins all increased. The expression of the above pathway proteins increased significantly (p<0.01) when the cells were irradiated, but zymosan pretreatment reduced the response of the above proteins to radiation. After that, the treatment of irradiated AHH-1 and HIEC cells with MyD88 inhibitor
ST2825 revealed that TLR2 and TLR4 protein expression was markedly increased (p<0.05), while MyD88, G-CSF, GM-CSF, IL-12 and IL-6 protein expressions were significantly reduced (p<0.05). This means that the radiation protection mechanism of zymosan is related to TLR2/4-MyD88-G-CSF/GM-CSF/IL-12/IL-6 pathway.

**Discussion**

Actually, humans are inevitably exposed to some radiation environments derived from various trace radionuclides such as cosmic rays, ground soil, buildings, and daily necessities, but these natural radiations rarely cause fatal radiation damage to human beings. However, the development and utilization of artificial radiation sources such as nuclear power stations, nuclear reactors, and nuclear weapons have forced mankind to face new life-threatening risks. Once there is a safety issue with nuclear sources, the generated ionizing radiation may cause fatal radiation damage to humans or other organisms [18]. In recent years, more and more attention has been paid to radiation related research in the world.

Nuclear radiation injury mainly refers to the destruction of genetic genes, biological macromolecular structures and the degeneration and necrosis of tissue cells caused by ionizing radiation, thus causing dysfunction of tissues and organs [19, 20]. The mechanism of the occurrence and development of radiation injury has been analyzed thoroughly, but no ideal treatment has been found. At present, there are two main ways to protect against ionizing radiation: one is external protection, that is, to reduce the damage of rays for the body by using appropriate shielding materials; the other is internal protection, to develop appropriate anti-radiation drugs. It is to perform radiation protection before possible radiation exposure or to treat radiation sickness after radiation exposure [21, 22]. Now most of the drugs used in the treatment of anti-radiation damage are western medicines, with large adverse reactions. They are mainly used for pre irradiation prevention and early treatment after irradiation, and are not suitable for long-term use, but the repair of the body after radiation damage requires a long-term treatment process. Therefore, it is imperative to search for the best anti-radiation drugs with high efficiency, low toxicity, small side effects, and the best route of oral administration as well as to explore the mechanism of radiation protection.

At present, some progress has been made in the research of anti-radiation drugs. The potency of sulfur-containing radiation preventive drugs (cysteamine S-phosphate sodium salt, N-acetylcysteine) is generally not high [23, 24]. The potency of vitamins and hormones is equivalent to that of sulfur-containing drugs, but their effective dose is thousands of times of physiological concentration, and the side effects of long-term use are not easy to overcome [25, 26]. Zymosan is a kind of natural polysaccharide β-glucan that is naturally present in the yeast cell wall, which has strong activity and no side effects. Zymosan has been shown to combine with receptors on the surface of immune cells to regulate immune responses and enhance the immune activity of cells [27]. Taghavi et al. also found that zymosan had anti-tumor activity, and played a positive role in inhibiting the progress of melanoma by regulating the expression of TLR-2, TLR-4 and TNF-α [28]. Although it has been reported that fungal beta
glucan and mushroom β-glucan can improve the survival rate of irradiated mice [29, 30], there is little research on the anti-radiation effect of zymosan. In this study, based on the cell level, the radiation protection effect of zymosan on AHH-1 and HIEC cells irradiated with different radiation time and different radiation doses was studied, and the mechanism of action was preliminarily explored. The results showed that zymosan was nontoxic to the cells, and the apoptosis rate of the cells pretreated by zymosan was significantly reduced, and the radiation protection effect is better than LPS. Furthermore, it was found that the mechanism was related to the activation of TLR2/4-MyD88/G-CSF/GM-CSF/IL-12/IL-6 pathway. The above results fully show the effectiveness of zymosan on the protection of ionizing radiation, indicating that it can be used for radiation related workers, such as medical radiation, nuclear accident personnel and other radiation safety protection, and it has potential application value.

However, the magnitude and severity of nuclear radiation's harm to human health also depend on factors such as the type of radiation, radiation dose rate, absorbed dose of the body and individual sensitivity. Different types of nuclear radiation have significant differences in relative biological effects on organisms. Therefore, in the future research, we will commit ourselves to further carry out the above issues, and provide basic biological data for the development of effective radiation protection drugs.

Conclusions

In summary, zymosan was nontoxic to the cells, and the radiation protection effect is better than LPS. The mechanism was related to the activation of TLR2/4-MyD88/G-CSF/GM-CSF/IL-12/IL-6 pathway.

Abbreviations

TLR (Toll-like receptors), pattern recognition receptors (PRR), nuclear factor kappa beta (NF-κB), myeloid differentiation factor 88 (MyD88), interleukin (IL), tumor necrosis factor (TNF), granulocyte colony-stimulating factor (G-CSF), recombinant human macrophage colony stimulating factor (M-CSF), granulocyte-macrophage colony stimulating factor (GM-CSF), lipopolysaccharide (LPS)

Declarations

Ethics approval and consent to participate

Not application.

Consent for publication

Not applicable

Availability of data and material

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.
Competing interests

The authors have no conflicts of interest to declare.

Funding

No.

Authors’ contributions

QZL and JJM analyzed and interpreted the western blot data. YZZ and SJG performed the examination of cells apoptosis, and was a major contributor in writing the manuscript. HCL performed the experiments of cells activity. All authors read and approved the final manuscript.

Acknowledge

No.

References

1. Vrijheid M, Cardis E, Blettner M, Gilbert E, Hakama M, Hill C, et al., The 15-Country Collaborative Study of Cancer Risk among Radiation Workers in the Nuclear Industry: Design, Epidemiological Methods and Descriptive Results. Radiation Research, 2007. 167(4): p. 361-379, 319.
2. Koning AJ and Rochman D, Towards sustainable nuclear energy: Putting nuclear physics to work. Annals of Nuclear Energy, 2008. 35(11): p. p.2024-2030.
3. Weinberg and A. M, Social Institutions and Nuclear Energy. Science, 1972. 177(4043): p. 27-34.
4. López PO, Dauer LT, Loose R, Martin CJ, Miller DL, Va?Ó E, et al., ICRP Publication 139: Occupational Radiological Protection in Interventional Procedures. Annals of the ICRP, 2018. 47(2): p. 1-118.
5. Millson C, Nuclear Weapons Testing in the United States: Sacrificing Health for National Defense. Student Pulse, 2011.
6. Cairnie AB, Adverse effects of radioprotector WR2721. Radiation research, 1983. 94(1): p. 221-226.
7. Medzhitov, Ruslan, Preston-Hurlburt, and Paula, A human homologue of the Drosophila Toll protein signals activation of adaptive immunity. Nature, 1997.
8. Kawai T and Akira S. TLR signaling. in Seminars in Immunology. 2007.
9. Aderem A and Ulevitch RJ, Toll-like receptors in the induction of the innate immune response. Nature, 2000. 406(6797): p. 782-787.
10. Ciorba MA, Riehl TE, Rao MS, Moon C, Ee X, Nava GM, et al., Lactobacillus probiotic protects intestinal epithelium from radiation injury in a TLR-2/cyclo-oxygenase-2-dependent manner. Gut, 2012. 61(6): p. 829-838.
11. Liu C, Zhang C, Mitchel RE, Cui J, Lin J, Yang Y, et al., A critical role of toll-like receptor 4 (TLR4) and its’ in vivo ligands in basal radio-resistance. Cell Death & Disease, 2013. 4(5): p. e649.
12. Xu W, Xiu S, Yang F, Ying H, Ruifeng Li, Dan X, et al., Protective Effect of Polysaccharides Isolated from Tremella fuciformis against Radiation-induced Damage in Mice. Journal of Radiation Research, 2012(3): p. 3.

13. Hassan AI, Ghoneim MAM, Mahmoud MG, Asker MMS, and Mohamed SS, Efficacy of polysaccharide from Alcaligenes xylosoxidans MSA3 administration as protection against γ-radiation in female rats. Journal of Radiation Research, 2015(2): p. 2.

14. Dobrek Z, Majewski S, and Maciejewski B, [Serum seromucoid levels in patients with neoplasms and in cases of radiation-induced necrosis following non-specific stimulation with zymosan]. Pol Tyg Lek, 1973. 28(48): p. 1886-1889.

15. Bremmer I and Garman C, [Experimental studies and therapeutic experiences on the radiation protection effect of subcutaneously and intramuscularly administered zymosan]. Radiobiologia Radiotherapia, 1961. 2(9): p. 129-134.

16. Bykhovskii AV, Komovnikov GS, and Polushkin BV, [Effects of zymosan on the macrophage reaction of the lungs and phagocytosis in acute radiation sickness]. Vestnik Akademii Meditsinskikh Nauk Sssr, 1965. 20(9): p. 83.

17. Federica C, Rosado MM, Simona C, Elia G, Silvia B, Marina V, et al., Pharmacological inhibition of TLR9 activation blocks autoantibody production in human B cells from SLE patients. Rheumatology, 2010(12): p. 12.

18. Sovacool BK, Questioning the Safety and Reliability of Nuclear Power: An Assessment of Nuclear Incidents and Accidents. Gaia Ecological Perspectives for Science & Society, 2011. 20(2): p. 95-103(109).

19. Coleman CN, Stone HB, Moulder JE, and Pellmar TC, Modulation of Radiation Injury. Science, 2004. 304(5671): p. 693-694.

20. LOBRICH, COOPER PK, RYDBER B, and M., Non-random distribution of DNA double-strand breaks induced by particle irradiation. International Journal of Radiation Biology & Related Studies in Physics Chemistry & Medicine, 1996. 70(5): p. 493-503.

21. Thiess A and Reizel C, Radiation protection material, method for production of a radiation protection material and use of the same. 2006.

22. Monje ML and Palmer T, Radiation injury and neurogenesis. Current Opinion in Neurology, 2003. 16(2): p. 129-134.

23. Ma SJ, Rivers CI, Serra LM, and Singh AK, Long-term outcomes of interventions for radiation-induced xerostomia:A review. World Journal of Clinical Oncology, 2019. 10(01): p. 4-16.

24. Landauer MR, Davis HD, Dominitz JA, and Weiss JF, Comparative Behavioral Toxicity of Four Sulfhydryl Radioprotective Compounds in Mice: WR2721, Cysteamine, Diethyldithiocarbamate, and N-Acetylcysteine. Pharmacology ? Therapeutics, 1988. 39(1-3): p. 97-100.

25. Srinivasan V, Weiss JF, and Kumar S, Radioprotection by misoprostol (PGE1 methyl analog) in combination with vitamin E, selenomethionine and WR-3689794. Advances in Experimental Medicine & Biology, 1997. 400B: p. 791-797.
26. Ghosh SP, Kulkarni S, Hieber K, Toles R, Romanyukha L, Kao TC, et al., Gamma-tocotrienol, a tocol antioxidant as a potent radioprotector. International Journal of Radiation Biology, 2009. 85(7): p. 598-606.

27. Dillon S, Agrawal S, Banerjee K, Letterio J, and Pulendran B, Yeast zymosan, a stimulus for TLR2 and dectin-1, induces regulatory antigen-presenting cells and immunological tolerance. Journal of Clinical Investigation, 2006. 116(4): p. 916-928.

28. Taghavi M, Mortaz E, Khosravi A, Vahedi G, Folkerts G, Varahram M, et al., Zymosan attenuates melanoma growth progression, increases splenocyte proliferation and induces TLR-2/4 and TNF-α expression in mice. Journal of Inflammation, 2018. 15(1): p. 5.

29. Pillai TG, Maurya DK, Salvi VP, Janardhanan KK, and Nair CKK, Fungal beta glucan protects radiation induced DNA damage in human lymphocytes. Annals of Translational Medicine, 2014. 2(2): p. 13.

30. Thulasi, G., Pillai, and, P., Uma, et al., Mushroom beta glucan: Potential candidate for post irradiation protection. Mutation Research/Genetic Toxicology and Environmental Mutagenesis, 2013.

**Figures**
Figure 1

The effect of zymosan on the G-CSF/GM-CSF/IL-12/IL-6 pathway of cells. (A) The effect of zymosan (20 µg/mL) on G-CSF, GM-CSF, IL-12 and IL-6 protein expressions in AHH-1 cells. (B) The effect of zymosan (20 µg/mL) on G-CSF, GM-CSF, IL-12 and IL-6 protein expressions in HIEC cells. *p<0.05, **p<0.01.
Figure 2

The effect of Zymosan on the TLR2/4-MyD88 pathway in cells. (A) The effect of zymosan (20 µg/mL) on TLR2, TLR4 and MyD88 protein expression in AHH-1 cells. (B) The effect of zymosan (20 µg/mL) on TLR2, TLR4 and MyD88 protein expression in HIEC cells. *p<0.05,**p<0.01.
Figure 3

Comparison of the protective effects of LPS and zymosan on cell radiation. (A) Effects of LPS and zymosan on AHH-1 cell activity after irradiation; (B) Effects of LPS and zymosan on HIEC cell activity after irradiation; (C) Effects of LPS and zymosan on the apoptosis rate of AHH-1 cells after irradiation; (D) Effects of LPS and zymosan on the apoptosis rate of HIEC cells after irradiation.
Figure 4

Study on radiation protection of zymosan for cells. (A) Effects of zymosan at different concentrations (0, 5, 10, 20 µg/mL) on the activity of irradiated AHH-1 cells; (B) Effects of zymosan at different concentrations on the activity of irradiated HIEC cells; (C) Effect of zymosan on apoptosis rate of irradiated AHH-1 cells; (D) Effect of zymosan on apoptosis rate of irradiated HIEC cells. Compared with Control group, **p<0.01; Compared with 0 µg/mL Zymosan, #p<0.05, ##p<0.01.
Figure 5

(A) The apoptosis rate of AHH-1 cells treated with different concentrations of zymosan for 72 h; (B) The apoptosis rate of HIEC cells treated with different concentrations of zymosan for 72 h.
Figure 6

(A) The apoptosis rate of AHH-1 cells treated with different concentrations of zymosan for 48 h; (B) The apoptosis rate of HIEC cells treated with different concentrations of zymosan for 48 h.
Figure 7

(A) The apoptosis rate of AHH-1 cells treated with different concentrations of zymosan for 24 h; (B) The apoptosis rate of HIEC cells treated with different concentrations of zymosan for 24 h.
Figure 8

The effect of zymosan on cell activity. (A) The activity of AHH-1 cells treated with different concentrations of zymosan for 24, 48 and 72 hours; (B) The activity of HIEC cells treated with different concentrations of zymosan for 24, 48 and 72 hours.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Additionalfiles.docx