Original Article

DOI: 10.7860/JCDR/2017/19367.9437

Radioprotective Effect of Beta D-Glucan and Vitamin E on Gamma Irradiated Mouse

Radiology Section

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ABSTRACT

Introduction: It is shown that beta-D-glucan is an immunologic system booster with radioprotectory effects. Radioprotectors are chemical components that can alleviate biological damage produced by ionizing radiation.

Aim: This study was designed to investigate the synergistic radioprotectory effects of beta-D-glucan and vitamin E on irradiated mice with ^{60}Co source.

Materials and Methods: A total of 240 female mice were arranged in four, equal population groups of control group (C), treated group with beta D-glucan (G), treated group with vitamin E (E), and treated group with both beta D-glucan and vitamin E (G+E). Each group was divided into three equal population groups of D6, D7 and D8 exposed to ^{60}Co radiation with prescribed total body dose of 6, 7 and 8 Gray (Gy), respectively. After the exposure, the number of survived animals was counted by time, then Lethal $Dose_{50/30}$ ($LD_{50/30}$), Lethal $Dose_{50/60}$ ($LD_{50/60}$) and Dose Reduction Factor (DRF) were calculated in all groups and corresponding groups.

Results: Based on the results of current study, treatment of the animals with vitamin E did not change values of $LD_{50/30}$ and $LD_{50/60}$, in comparison to control group. $LD_{50/30}$ and $LD_{50/60}$ of treated groups with beta D-glucan and beta D-glucan + vitamin E showed significant difference with those of control group ($p<0.01$). The DRF values in groups E, G and G + E, were calculated respectively as 1, 1.25 and 1.375 based on $LD_{50/30}$, and respectively as 1, 1.17 and 1.33 based on $LD_{50/60}$. While values of DRF in groups G and G + E showed significant difference in comparison to that of control group ($p<0.01$), but the difference between DRF of groups G and G + E was not significant ($p=0.395$).

Conclusion: The findings of study obviously showed that, presence of beta D-glucan in the body of mice, during exposure to ionizing radiation, leads to DRF of higher than one, proving the radioprotectory effect of this agent. Also, we demonstrated that, while vitamin E had no radioprotectory effect on irradiated mice, beta D-glucan in combination with vitamin E increased resistance of mice against ionizing radiation.

Keywords: Ionizing radiation, Radioprotectors, Toxicity

INTRODUCTION

Radioprotectors are chemical components that can alleviate biological damage produced by ionizing radiation. The first evidence of agents that were used as radioprotectors to reduce harmful effect of radiation on the mammalian bodies dates back to several decades ago [1]. Radioprotectors can have a wide range of applications. For radiation technologists or those who may be subject of unwanted exposure to ionizing radiation, application of radioprotectors could be used with radiation protection purpose to effectively reduce biological side effects. In patients who undergo radiotherapy procedures as a treatment, absorption of high level of radiation may lead to post therapy complications in their blood, intestine and immunologic systems. Radioprotectors, here, may be used as an effective agent that could allow application of high dose to the tumour with minimum risk to normal tissues. Adverse effects of ionizing radiation in both groups, irradiated staff and treated patients with radiation, can be reduced using radioprotectors.

Up till now, several attempts have been done to introduce and study components that exhibit radioprotective properties [2-7], none of which meet the ideal radioprotector requirements and act perfectly. Most of the radioprotectors have cysteine amino acid and cysteine amine in their structures that are capable of blocking performance of free radicals in the body, however a vast majority of effective radioprotectors are toxic and there are continuous researches conducting to introduce new suitable radioprotectors with respect to properties such as efficiency, toxicity, manufacturing costs and duration of stability. One of these substances with potential radioprotective properties is beta D-glucan. Beta D-glucan is a polysaccharide which acts as an immune system booster and may improve recovery of patients after radiation therapy [8,9]. This agent is shown to have a good antioxidant activity by scavenging of hydroxyl radical [10]. It is also reported that, beta glucan of *Ganoderma lucidum* have significant DNA repairing ability [11]. This chemical component can reinforce immune system and protect body against ionizing radiation [12].

Molecular structure and chain conformations of D-glucan is well studied using infrared spectroscopy, Nuclear Magnetic Resonance (NMR) and laser scattering [13]. It is shown that, D-glucan is composed of main chain of 1-3 beta D-glucan and side chain of 1-6 beta-D glucopyranosyl, and can be extracted from mushroom, bacteria and plant [14,15]. Because of interesting properties of this component including anti-tumour and anti-osteoporosis effects, improvement of the immune system [13] and anti-infection properties [16,17], beta D-glucan, has drawn interest of researches as an effective radioprotector agent.

Similar to D-glucan, vitamin E is also known as anti-oxidant agent that can decrease radiation induced damage in vitro [18]. This agent is shown to reduce chromosomal aberrations as well as micronuclei in bone marrow of irradiated mouse with 1 Gy [19]. However, there is also evidence in which vitamin E has not protected chromosomes against X-ray photons [20].

Therefore, in current study we assessed radioprotectory effect of beta D-glucan, alone and in combination with vitamin E, on irradiated mice with gamma photons, using Dose Reduction Factor, Lethal $Dose_{50/30}$ ($LD_{50/30}$), Lethal $Dose_{50/60}$ ($LD_{50/60}$).

MATERIALS AND METHODS

A total of 240 female mice were arranged in four, equal population, groups of control (C), treated with beta D-glucan (G), fed with 250

mg/body-weight, treated with vitamin E (E), fed with 1 mg/body-weight for one month, and treated with both beta D-glucan and vitamin E (G+E). Each group was then divided into three equal population groups of D6, D7 and D8 exposed to ^{60}Co radiation with prescribed total body dose of 6, 7 and 8 Gy, respectively.

To assess response of the animals to ionizing radiation, three quantities of $LD_{50/30}$, $LD_{50/60}$ and DRF were calculated for each of groups. $LD_{50/30}$ is a dose of radiation that can kill half of the irradiated animals within 30 days. Similarly, $LD_{50/60}$ refers to the dose of radiation that can kill half of the animals within 60 days. DRF is another quantity to describe the effectiveness of a radioprotector and is defined as the ratio of the dose required to cause death in the presence of the component divided by the dose required to create the same effect in the absence of component.

STATISTICAL ANALYSIS

The analysis of variance (Kruskal–Wallis test) was used for each dose under four treatments. A p-value of less than 0.05 was considered to be statistically significant. The SPSS for Windows software package (Release 22, SPSS Inc., Chicago, Illinois) was applied for the statistical analysis.

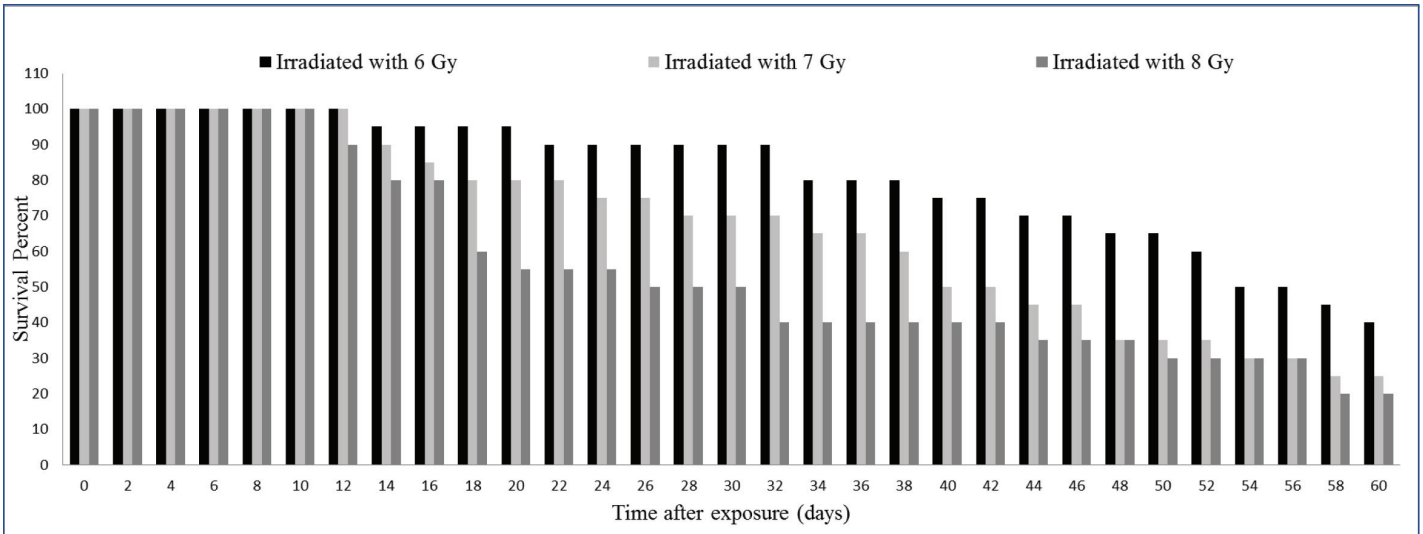
RESULTS

Surviving rate of animals, after exposure to the radiation doses of 6, 7 and 8 Gy, in control group and treated groups with vitamin E, beta D-glucan and vitamin E + beta D-glucan are given in [Table/Fig-1-4]. DRF of these groups, with respect to the $LD_{50/30}$ and $LD_{50/60}$, were also given in [Table/Fig-5]. In [Table/Fig-6], $LD_{50/30}$ and $LD_{50/60}$ of the control and treated groups are presented.

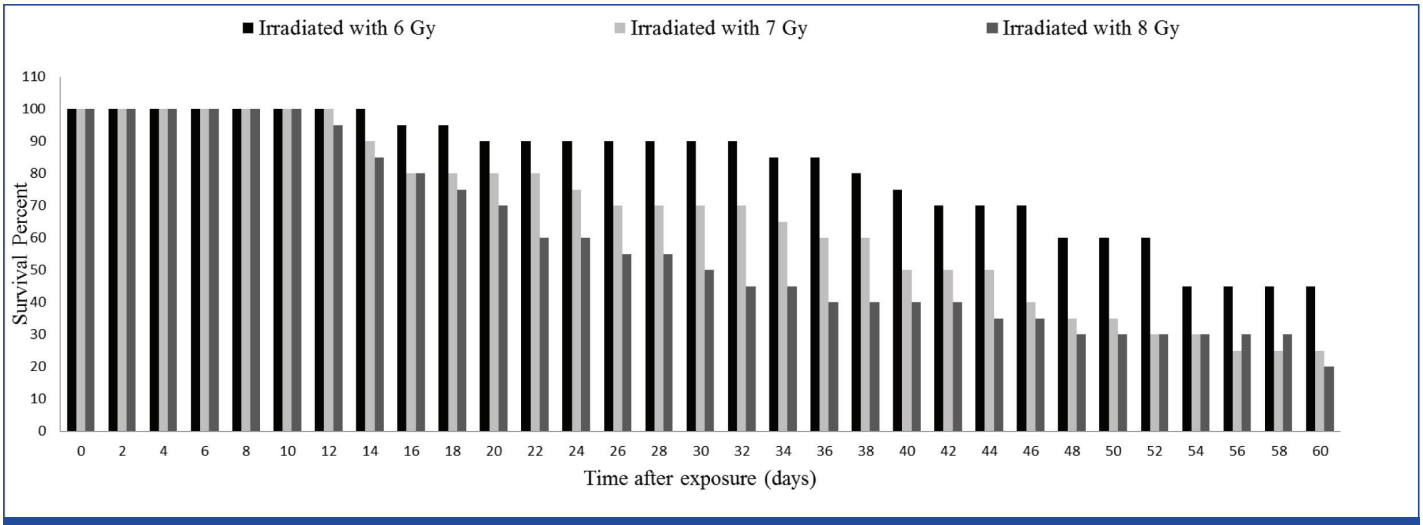
Based on the statistical analysis of the results;

- The difference of DRF in group G in comparison with group C was significant at both $LD_{50/30}$ (p= 0.005) and $LD_{50/60}$ (p= 0.005).
- The difference of DRF in group E in comparison with group C was insignificant at both $LD_{50/30}$ (p= 1.000) and $LD_{50/60}$ (p=1.000).
- The difference of DRF in group E + G in comparison with group C was significant at both $LD_{50/30}$ (p≤ 0.001) and $LD_{50/60}$ (p≤ 0.001), but in comparison with group G it was insignificant at both $LD_{50/30}$ (p=0.395) and $LD_{50/60}$ (p=0.395).

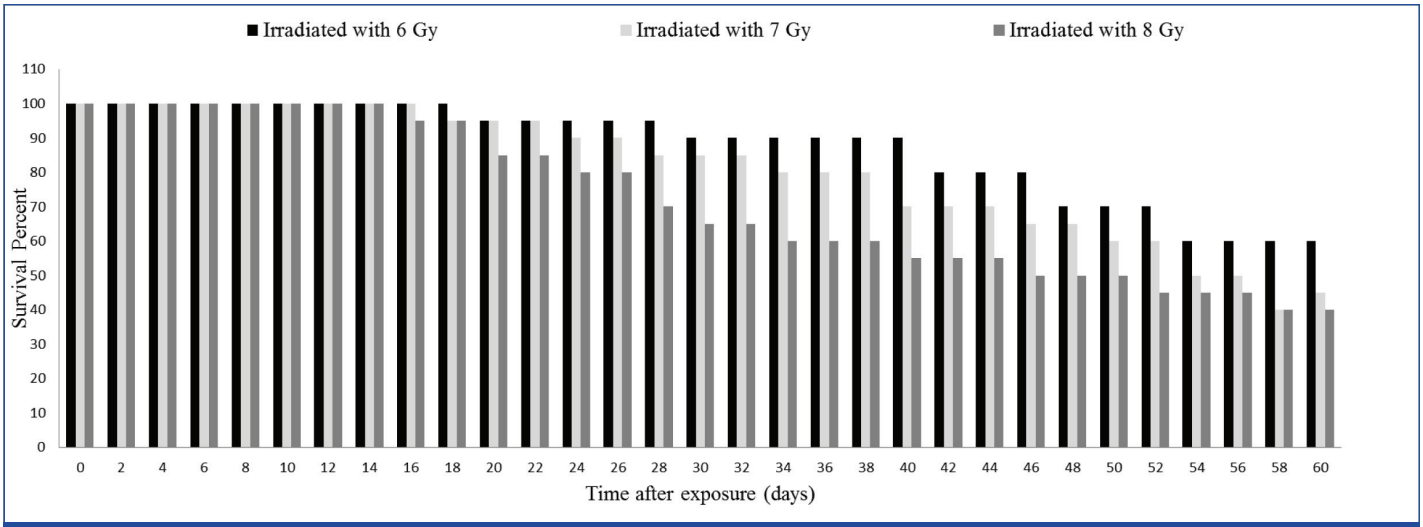
According to [Table/Fig-6], in control group, $LD_{50/30}$ and $LD_{50/60}$ are 8 and 6 Gy, respectively. Values of $LD_{50/30}$ and $LD_{50/60}$ in the group treated with vitamin E were same as that of control group, which indicate that vitamin E, alone, did not have effect on the animal resistance against ionizing radiation. For groups G and G + E of animals, we observed that, at the end of 30 days, number of animals killed by radiation did not reached to the ratio of 50%, therefore, we had to increase radiation doses up to 9, 10 and 11 Gy. The $LD_{50/30}$ values for group G and group G + E was found 10 Gy and 11 Gy, respectively. Both findings show significant difference with that of control group (p<0.001), which means that both beta D-glucan and combination of beta D-glucan with vitamin E has radioprotective effect on animals. Any difference between these two groups was not observed. Also, after exposure of animals of group G and G + E to dose of 6 Gy, half of the animals did not die within the 60 days and it took 66 days to reach to this number. For these groups, $LD_{50/60}$ was determined 7 Gy for group G and 8 Gy for group G + E, both were significantly higher than that of control group (p<0.005).



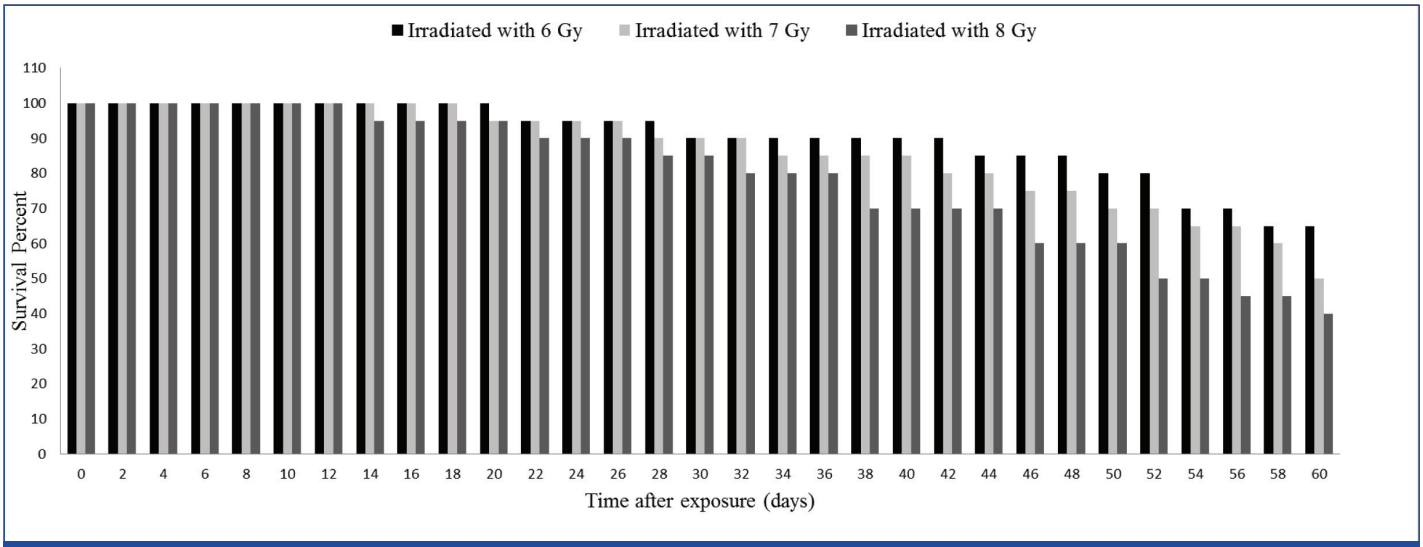
[Table/Fig-1]: Survival percent in group C irradiated at 6, 7 and 8 Gy doses of ^{60}Co over the 60 day follow up.



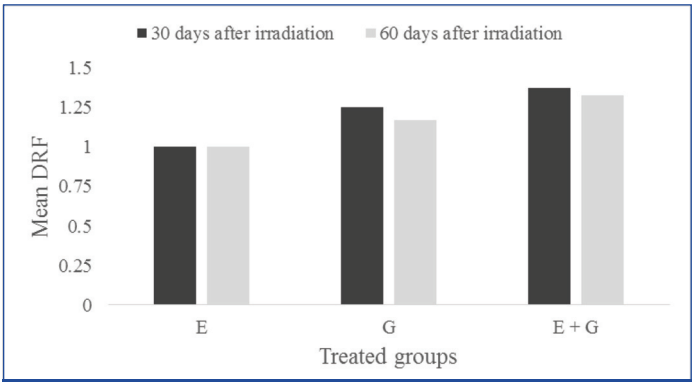
[Table/Fig-2]: Survival percent in group E irradiated at 6, 7 and 8 Gy doses of ^{60}Co over the 60 day follow up.



[Table/Fig-3]: Survival percent in group G irradiated at 6, 7 and 8 Gy doses of ^{60}Co over the 60 day follow up.



[Table/Fig-4]: Survival percent in group E + G irradiated at 6, 7 and 8 Gy doses of ^{60}Co over the 60-day follow up.



[Table/Fig-5]: Values of Dose Reduction Factor (DRF) for treated groups of E, G and E + G with respect to $LD_{50/30}$ and $LD_{50/60}$.

Group	$LD_{50/30}$ (Gy)	DRF	$LD_{50/60}$ (Gy)	DRF
Control (C)	8	-	6	-
Vitamin E (E)	8	1	6	1
Beta Glucan (G)	10	1.25	7	1.17
Beta D-Glucan + Vitamin E (G+E)	11	1.375	8	1.33

[Table/Fig-6]: Values of $LD_{50/30}$ and $LD_{50/60}$ in control group and three treated groups.

DISCUSSION

These finding was another evidence to confirm that, beta D-glucan and its combination with vitamin E exhibit radioprotective effect. Our results agree with previous findings in which radioprotective properties of beta-glucan on immune system of the body was studied [14,17,21]. In a study, anti-tumour and radioprotectory properties of beta D-glucan was measured on mice, based on the tumour volume, Natural Killer (NK) and Lymphokine Activated Killer (LAK) [22]. It was shown in treated group with beta D-glucan, weight and survival rate of animals not just had significant difference with those of control group, but level of NK and LAK was also increased. In other similar study, it was reported that chronic treatment with beta D-glucan decreased growth rate of tumour [23]. The reason of increased radioprotectory effect after administration of beta D-glucan was mentioned the hematopoietic effect as a result of increase of leukocytes and lymphocytes. In another attempt, anti-tumour effect of beta D-glucan in combination of vitamin C on breast and lung tumours was reported [24]. Authors of that study concluded that simultaneous presence of these two components as wells as anti-tumour effect of beta D-glucan had been the reason for that finding. Synergistic effect of beta D-glucan in combination of resveratrol on the immune system has also been reported in similar studies [25,26].

Of the main findings of current study was the synergistic effect of beta D-glucan in combination with vitamin E to increase body radioprotective capability, which is in a good agreement with previous studies [21,26]. On the other hand, DRF of group G + E was higher than that of group E, which means beta D-glucan increased radioprotective effect of vitamin E. However, DRF of group G + E was not significantly higher than that of group G.

Because of the effectiveness of beta D-glucan and its combination with vitamin E as a radioprotective agent, this component could be used in clinical context for the patients that, because of treatment operations, their immune systems have been weakened. However,

comprehensive studies are required to translate this compound from bench to beside. For this reason, we conducted another research project to assess effect of beta D-glucan in reduction of side effects of ionizing radiation on healthy tissues of patients undergoing radiation based therapy. Also, because of the positive effect of beta D-glucan on the immune system, another study was planned to evaluate the effect of this substance on mitigation of chemotherapy side effects.

LIMITATION

In this attempt we used a fixed dose of beta D-glucan at 250 mg/body weight that is a limitation to this study, therefore, it is recommended to assess a similar study at different doses of this component in order to reach to an optimum dose of beta D-glucan as a radioprotective agent in combination with vitamin E.

CONCLUSION

The findings obviously demonstrated that, presence of beta D-glucan in the body of mice, during exposure to ionizing radiation, leads to DRF of higher than one, proving the radioprotectory effect of this agent. Also, we depicted that, while vitamin E had no radioprotectory effect on irradiated mice, beta D-glucan in combination with vitamin E increased resistance of mice against ionizing radiation.

REFERENCES

[1] Gudkov SV, Popova NR, Bruskov VI. Radioprotectors: History, trends and prospects. Biofizika. 2015;60(4):801-11.
[2] Burdak-Rothkamm S, Smith A, Lobachevsky P, Martin R, Prise KM. Radioprotection of targeted and bystander cells by methylproamine. Strahlenther Onkol. 2015;191(3):248-55.
[3] Smith PJ, Anderson CO. Modification of the radiation sensitivity of human tumour cells by a bis-benzimidazole derivative. International Journal of Radiation Biology and Related Studies in Physics, Chemistry, and Medicine. 1984;46(4):331-44.
[4] Stone HB, Moulder JE, Coleman CN, Ang KK, Anscher MS, Barcellos-Hoff MH, et al. Models for evaluating agents intended for the prophylaxis, mitigation and treatment of radiation injuries. Report of an NCI Workshop, December 3-4, 2003. Radiation research. 2004;162(6):711-28.
[5] Theriot CA, Casey RC, Moore VC, Mitchell L, Reynolds JO, Burgoyne M, et al. Dendro {C(60)} fullerene DF-1 provides radioprotection to radiosensitive mammalian cells. Radiation and Environmental Biophysics. 2010;49(3):437-45.
[6] Weiss JF, Landauer MR. History and development of radiation-protective agents. International Journal of Radiation Biology. 2009;85(7):539-73.
[7] Citrin D, Brown A, Chung E, Urick M, Shield W, Sowers A. Evaluation of the fullerene compound DF-1 as a radiation protector. Radiat Oncol. 2010;5.
[8] Chen J, Seviour R. Medicinal importance of fungal beta-(1-->3), (1-->6)-glucans. Mycological Research. 2007;111(Pt 6):635-52.
[9] Yamamoto K, Kimura T, Sugitachi A, Matsuura N. Anti-angiogenic and anti-metastatic effects of beta-1,3-D-glucan purified from Hanabiratake, Sparassis crispa. Biological & Pharmaceutical Bulletin. 2009;32(2):259-63.
[10] Pillai TG, Uma Devi P. Mushroom beta glucan: Potential candidate for post irradiation protection. Mutation Research. 2013;751(2):109-15.
[11] Pillai TG, Maurya DK, Salvi VP, Janardhanan KK, Nair CK. Fungal beta glucan protects radiation induced DNA damage in human lymphocytes. Annals of Translational Medicine. 2014;2(2):13.
[12] Tada R, Tanioka A, Iwasawa H, Hatashima K, Shoji Y, Ishibashi K, et al. Structural characterisation and biological activities of a unique type beta-D-glucan obtained from Aureobasidium pullulans. Glycoconjugate Journal. 2008;25(9):851-61.
[13] Wang J, Zhang L. Structure and chain conformation of five water-soluble derivatives of a beta-D-glucan isolated from Ganoderma lucidum. Carbohydrate Research. 2009;344(1):105-12.
[14] Akramiene D, Kondrotas A, Didziapetriene J, Kevelaitis E. Effects of beta-glucans on the immune system. Medicina. 2007;43(8):597-606.
[15] Weitberg AB. A phase I/II trial of beta-(1,3)/(1,6) D-glucan in the treatment of patients with advanced malignancies receiving chemotherapy. Journal of Experimental & Clinical Cancer Research. 2008;27:40.
[16] Dennehy KM, Brown GD. The role of the beta-glucan receptor Dectin-1 in control of fungal infection. Journal of Leukocyte Biology. 2007;82(2):253-58.
[17] Presterl E, Parschalk B, Bauer E, Lassnigg A, Hajdu S, Graninger W. Invasive fungal infections and (1,3)-beta-D-glucan serum concentrations in long-term intensive care patients. International Journal of Infectious Diseases. 2009;13(6):707-12.
[18] Borek C, Ong A, Mason H, Donahue L, Biaglow JE. Selenium and vitamin E inhibit radiogenic and chemically induced transformation invitro via different mechanisms. Proceedings of the National Academy of Sciences of the United States of America. 1986;83(5):1490-94.
[19] Sarma L, Kesavan PC. Protective effects of vitamins C and E against gamma-ray-induced chromosomal damage in mouse. International Journal of Radiation Biology. 1993;63(6):759-64.
[20] Umegaki K, Itoh T, Ichikawa T. Effect of vitamin E on chromosomal damage in bone marrow cells of mice having received low dose of X-ray irradiation. International Journal for Vitamin and Nutrition Research. 1994;64(4):249-52.
[21] Yoshimura Akinobu MH, Hitoshi T, Tooru T. Anti-tumour and radiation protection effects of. BETA.-1 3-D-Glucan extracted from yeast (*Saccharomyces cerevisiae*). Radioisotopes. 2003;52(12):687-91.
[22] Chan GC, Chan WK, Sze DM. The effects of beta-glucan on human immune and cancer cells. Journal of Hematology & Oncology. 2009;2:25.
[23] Gu YH, Takagi Y, Nakamura T, Hasegawa T, Suzuki I, Oshima M, et al. Enhancement of radioprotection and anti-tumour immunity by yeast-derived beta-glucan in mice. Journal of Medicinal Food. 2005;8(2):154-58.
[24] Vetvicka V, Vetvickova J. Combination of glucan, resveratrol and vitamin C demonstrates strong anti-tumour potential. Anticancer Research. 2012;32(1):81-87.
[25] Vetvicka V, Vancikova Z. Synergistic effects of glucan and resveratrol. Afr J Biochem Res. 2010;4:105-10.
[26] Vetvicka V, Volny T, Saraswat-Ohri S, Vashishta A, Vancikova Z, Vetvickova J. Glucan and resveratrol complex possible synergistic effects on immune system. Biomedical papers of the Medical Faculty of the University Palacky, Olomouc, Czechoslovakia. 2007;151(1):41-46.

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FINANCIAL OR OTHER COMPETING INTERESTS: None.

Date of Submission: **Feb 07, 2016**
Date of Peer Review: **May 14, 2016**
Date of Acceptance: **Oct 14, 2016**
Date of Publishing: **Feb 01, 2017**