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FUNGAL POLYSACCHARIDE PROTECTS HUMAN LYMPHOCYTES FROM RADIATION INDUCED DAMAGE

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ABSTRACT

The radioprotective properties of polysaccharides isolated from the macro fungi Ganoderma lucidum was assessed by Single cell gel electrophoresis (Comet assay).Human lymphocytes were exposed to 0Gy, 2 Gy and 4 Gy gamma radiation in the presence and absence of polysaccharides. The comet parameters - % DNA, Tail length, Tail moment and olive tail moment were reduced by the presence of polysaccharides. The results indicate that the polysaccharides of G. lucidum possessed significant radioprotective activity. The findings suggest the potential use of this mushroom for the prevention of radiation induced cellular damages.

KEY WORDS

Medicinal mushroom, Ganoderma.lucidum, Polysaccharides, Comet assay, Radioprotection.

INTRODUCTION

Ganoderma, commonly known as Reishi has been considered as a panacea for all types of diseases. Reishi has attracted significant attention in recent years due to its large number of pharmacological properties [1]. The fruiting bodies of this mushroom contain a variety of chemical substances. Recent investigations carried out in our laboratory have shown that aqueous extract of *G.lucidum* possessed significant radio protective properties [2].

Radiation protection has significant importance in radiotherapy of cancer, nuclear accidents and even in nuclear warfare. Radiation induced cell damage results from either damage to cell membrane or DNA [3-4]. Lesions in DNA can be induced either by direct ionization of DNA or indirectly through the reaction of aqueous free radicals leading to base damage, intra or inter strand cross-linking and single or double strand breaks [3,5]. Protection of normal tissues against this cellular damage is important in radiotherapy. The major problem associated with cancer radiotherapy is the severe side effects and damage to normal tissue.

Ionizing radiation is one of the well-established and widely used therapeutic modalities either for curative or palliative treatment of tumors in man. In radiotherapy of cancer, normal tissues need to be protected while cancers are exposed to high doses of radiation. A large number of compounds natural and synthetic have been evaluated for this purpose [6]. However most of them were not successful clinically because of toxicity and side

effects. Hence search for an ideal radioprotector without side effects is a compelling urgency. In the present study we examined the radioprotective effect of the polysaccharides from *G.lucidum.*

MATERIALS AND METHODS

Chemicals

Tris base, high melting agarose, low melting point agarose, Na_2-EDTA, TritonX-100, sodium sarcosinate, DMSO and propidium iodide were obtained from Sigma Chemicals (St.Louis, Missouri). All other chemicals used were of analytical grade and procured locally.

Collection of human blood:

Human blood samples were collected from three healthy nonsmoking volunteers, having a mean age of 25±2 years.

Irradiation

^{60}Co-gamma rays in a Gamma Cell 220 (AECL, Canada) at a dose rate of 5.3Gy/ min and Junoir Theratron unit (AECL, Ottawa, Canada) with a dose rate of approximately 0.35Gy/min at 38 cm was used for irradiation purpose.

Isolation of polysaccharides

The fruiting bodies of *G.lucidum* were collected from the outskirts of Thrissur district, Kerala. The type specimen was deposited in the herbarium of Centre for Advanced Studies in Botany, University of Madras, Chennai, India (HERB. MUBL. 3175). Sporocarps were cut into small pieces, dried at 40-50°C for 48 hours and powdered. Polysaccharides were isolated by method of Mizuno [7] with slight modification The powdered sporocarps were defatted with petroleum ether and extracted with double distilled water at 80^{0}C for 8-10 hours in several batches. The extract were combined, filtered, and concentrated to about one third of the original volume and chilled ethanol about 5 times the original volume was added and kept at 4^{0}C for 48 hrs. The precipitate was collected after centrifugation, redissolved in distilled water and treated with Sevags reagent [8] several times to remove protein and then dialyzed against deionised water for 48 hrs at 4^{0}C. The dissolved precipitate were again precipitated with ethanol and the precipitate thus obtained was lyophilized to obtain the polysaccharides. Confirmation of the polysaccharides was done by Anthrone [9] and Phenol sulphuric acid tests [10].

Comet assay

Alkaline single-cell gel electrophoresis: Alkaline single-cell gel electrophoresis was performed using the method of Singh (2000) [11] with minor modifications [12-13]. In order to estimate DNA damage in blood leukocytes, 10µl heparinised whole blood was mixed with 200µl of low melting point agarose at 37°C and layered on frosted slides pre-coated with 200µl high melting point agarose. After solidification of agarose, the cover slips were removed and the slides were kept in pre-chilled lysing solution containing 2.5M NaCl, 100mM Na_2-EDTA: pH10.0, 10mM Tris HCl, 1% sodium sarcosinate with freshly added 1% Triton X-100 and 1% DMSO at 4°C for 1 hour. The slides were removed from the lysis solution and placed on a horizontal electrophoresis tank filled with the alkaline buffer (300mM NaOH, 1mM Na_2-EDTA, 0.2%DMSO, pH >13.0). The slides were equilibrated in the same buffer 20 minutes. Electrophoresis was carried out for 20 minutes at 25 V, 300mA using a compact power supply. After electrophoresis, the slides were stained by layering on the top with 50µL of propidium iodide (20µg/ml) and visualized using a Carl Ziess Axioskop microscope with bright field, phase contrast and epi-fluorescence facility (HBO 50 high pressure mercury lamp), 40X camera adaptor lens. The integral frame grabber used in his system (Cvfb01p) was a PC based card made in the Electronics Division of Bhabha Atomic Research Centre, and it accepted color composite video output of the camera.

The quantitation of the DNA strand breaks of the stored images was done using the imaging software CASP by which the percentage DNA in tail, tail length, tail moment, and Olive tail moment could be obtained directly [13]. The tail length of comet indicated the extent of damage because the smaller molecules moves faster on the agarose gel. Thus, the longer tails of the comets indicated that the strand breaks were more frequent and the DNA was fragmented into several small molecules. The tail moment was a commonly accepted unit of DNA damage that normalizes the difference in the size of the nucleus studied (e.g., blood leukocytes) [12-13]. It is the product of the percent DNA in the tail of the comet and tail length. For olive tail moment, distance of center of gravity of DNA is considered instead of usual tail length.

RESULTS

An exposure of human peripheral blood leukocytes to 0 Gy and 4Gy γ-radiation *ex vivo* resulted in increase of comet parameters such as % DNA in tail, tail length, tail moment and olive tail moment and the presence of polysaccharides at 500 µg/ml during irradiation reduced these parameters. (**Figure.1 and Table.1**). These results thus suggest a protection of cellular DNA by *G.lucidum* polysaccharides from radiation damage. The Olive tail moment at 2 Gy was reduced by polysaccharide from 2.6724±0.0881 to 1.8314±0.2041. Similarly at 4 Gy the Olive tail moment was reduced from 9.1036 ± 0.9719 to 7.1745 ± 0.1434.

Figure 1: Protection of human blood leucocytes against gamma radiation-induced strand breaks by polysaccharides isolated from *Ganoderma lucidum* (500µg/ml)

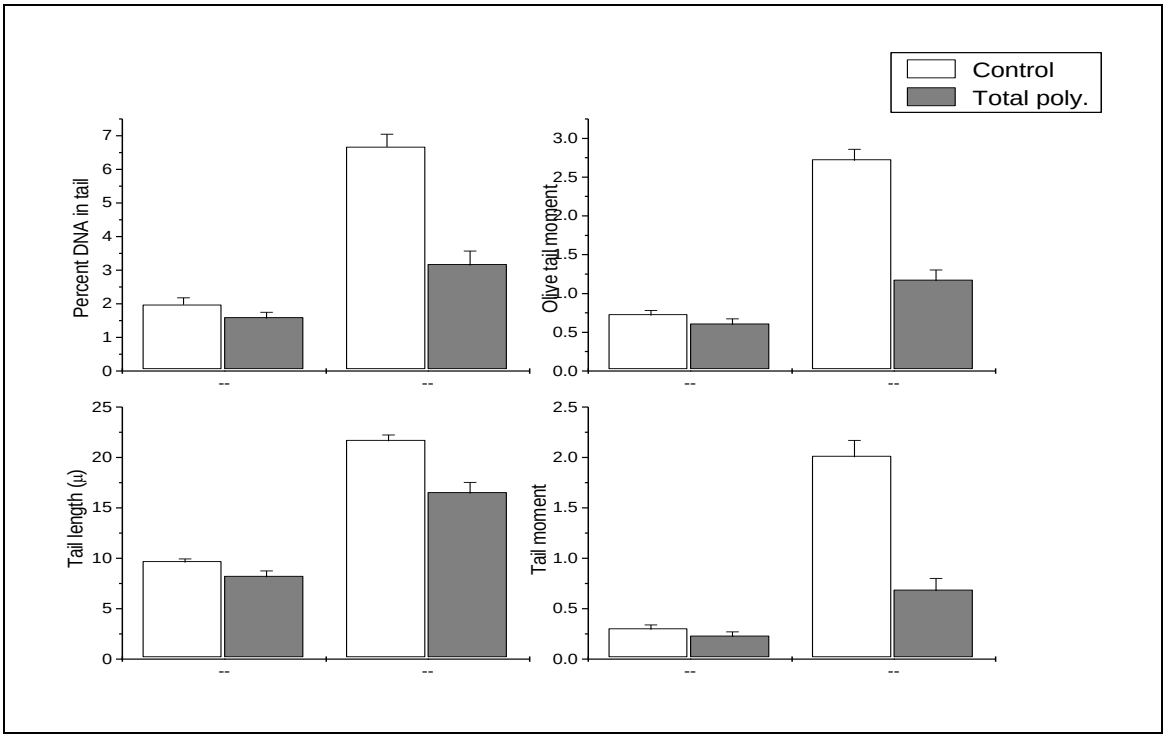

Table 1: Comet parameters in the presence and absence of polysaccharides at different doses of gamma irradiation.

Treatment	0 Gy	4 Gy
	% DNA	
Control	1.9718±0.21096	6.6679±0.3743
500 µg/ml	1.5941±0.1947	3.1772±0.4270
	Tail length	
Control	9.7137±0.3456	21.7296±0.6288
500 µg/ml	8.2476±0.6154	16.5423±1.1118
	Tail moment	
Control	0.3051±.0.0463	2.0148±0.1665
500 µg/ml	0.2324±0.0501	0.6887±0.1243
	Olive tail moment	
Control	0.7326±0.0637	2.7278±0.14507
500 µg/ml	0.3617±0.0691	1.1745±0.1434

DISCUSSION

DNA constitutes the primary vital target for cellular inactivation of living systems by ionizing radiation. Ionizing radiation damage to cellular DNA are mainly strand breaks, elimination of bases and sugar damage. Exposure of human leucocytes to 4Gy radiation cause severe damage as is reflected from the comet assay. The polysaccharides from the mushroom, *G. lucidum* causes decrease in the comet attributes. Administration of *G. lucidum* extract has been reported to provide significant relief from the side effects of chemotherapy [14].

The damages by ionizing radiation to DNA can cause the loss of viability of the cells exposed to radiation. The alkaline comet assay is an elegant and effective technique to monitor the extent of the DNA damage and its protection. One of the deleterious consequences of DNA damage from exposure to ionizing radiation is the induction of cancer. Protecting cellular DNA from radiation damage might result in the prevention of the cancers induced by the radiation. Fungal polysaccharides of comparable structure and function as those found in *Ganoderma* have undergone rigorous clinical trials and based on such indirect experimental evidence, it is hypothesized that this medicinal mushroom polysaccharide might render significant relief from the side effects of both chemotherapy and radiotherapy. Polysaccharides from *G.lucidum* also possess DNA repairing ability in human lymphocytes [15].

The result of present investigation reveals the potentials of *G.lucidum* in radiation protection not only in radiotherapy but also in accidental radiation exposure. The findings also suggest the possibility of using this medicinal mushroom polysaccharides as adjunct therapy in cancer radiotherapy.

REFERENCES

1. Tim L, Yihuai G., Shufeng Z. Global marketing of medicinal Ling Zhi mushroom *G.lucidum* products and safety concerns, Int. J. Med. Mushr, 6:189 – 194,(2004).
2. Thulasi G.P, Salvi V. P., Maurya D.K., Nair. C.K.K. and Janardhanan K.K. Prevention of radiation induced damages by aqueous extract of *Ganoderma lucidum* occurring in Southern parts of India. Current Science, 91: 341-344, (2006).
3. Haimovitz-Friedman, A. Radiation-induced signal transduction response, Radiat Res, 150:102 – 108, (1998).
4. Jonathan, E.C., Bernhard, E. J. and Mckenna, W. G. How does radiation kill cells? Curr Opin Chem Biol, 3: 77 – 83, (1999).

5. Ross, G.M., Induction of cell death by radiotherapy, Endocr Relat Cancer, 6:41 – 44, (1999).
6. Nair,C.K.K., Parida,D.K., Nomura,T. Radiation protectors in radiotherapy J.Radiat.Res., 42: 21-37(2001).
7. Mizuno, T. Development of an antitumor biological response modifier from Phellinus linteus Teng. (Review). Int.J. Med.Mushrooms. 21-33, (2000).
8. Staub, A.M. Removal of protein, Sevag method. Methods in carbohydrate Chemistry, 5: 5-6, (1965).
9. Yemn, E.W., Wills, A.J. The estimation of carbohydrate in plant extract by anthrone. Biochem. J. 57: 508-514, (1954).
10. Dubois, S.M.,Gilles, G.A., Hamilton, J.K. Colorimetric estimation of Carbohydrates by Phenol Sulphuric acid method, Anal.Chem, 28: 350-356, (1956)
11. Singh,N.P. Microgel for estimation of DNA strand breaks, DNA protein crosslinks and apoptosis. Mutat Res. 455:111-127, (2000).
12. Maurya, D.K., Salvi, V.P, Nair,C.K.K.,. Radioprotection of Normal Tissues in Tumour-bearing Mice by Troxerutin. J. Radiat. Res. 45: 221-228, (2004).
13. Konca, K., Lankoff, A., Banasik. A., Lisowska, H., Kuszewski, T., Gozdz, S., Koza, Z., Wojcik, A.,. A cross platform public domain PC image analysis program for the comet assay. Mutat. Res. 534: 15-20, (2003).
14. Shi, J.H. PSP for the protection of the tumor patients during chemotherapy. In PSP *Intl Symposium,* Yang Q Y and Kwok C Y (eds), Fudan U, Press, Shanghai, 271-272, 1993.
15. Thulasi G.P., Nair C.K.K., Janardhanan K.K. Enhancement of repair of radiation induced DNA strand breaks in human cells by *Ganoderma* mushroom polysaccharides, Fd. Chem. 119:1040-1043, (2009).

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