HAYATI Journal of Biosciences April 2015
Vol. 22 No. 2, p 93-97
EISSN: 2086-4094

Available online at:
http://journal.ipb.ac.id/index.php/hayati
DOI: 10.4308/hjb.22.2.93

SHORT COMMUNICATION

Lack of Radioprotective Potential of Ginseng in Suppressing Micronuclei Frequency in Human Blood Lymphocyte under Gamma Irradiation

YANTI LUSIYANTI*, ZUBAIDAH ALATAS, MUKH SYAIFUDIN

Center for Technology of Radiation Safety and Metrology, National Nuclear Energy Agency,
Jalan Lebakbulus Raya No. 49, Pasar Jum'at, Jakarta 12440, Indonesia

Received April 8, 2014/Accepted January 13, 2015

Ginseng appears to be a promising radioprotector for therapeutic or preventive protocols capable of attenuating the deleterious effects of radiation on human normal tissue. This research addresses results on the study of radioprotective potential of ginseng on radiation induced micronuclei in lymphocyte cells *in vitro*. The peripheral blood samples were exposed to gamma rays at doses of 0.0, 0.5, and 1.0 Gy and then added with 0, 100, and 1000 ug/mL of ginseng extract. These treated samples were cultured for micronuclei (MN) examination using standard procedure. The evaluation of incubation with ginseng extract for 24 h before irradiation was also done. Our results showed that there was no radioprotective effect of ginseng addition to the frequency of MN in lymphocyte cells. Pre-incubation with ginseng extract before irradiation also did not effectively suppress the MN frequency. This research lacks to prove the ginseng's radioprotective potential that maybe related to its immunomodulating capabilities and its capabilities in scavenging free radicals induced by radiation or in attenuating the deleterious effects of radiation and its important role in increasing levels of several cytokines.

Keywords: gamma rays, ginseng, micronuclei, radioprotective effects

INTRODUCTION

Although efforts had been directed to mitigate radiation-induced normal tissue damages since the discovery of the deleterious effects of radiation, the expanding role of radiotherapy in cancer treatment creates new imperatives for developing safe and effective agents for prophylaxis and treatment of ionizing radiation-induced tissue damage (Coleman *et al*. 2004). Many radioprotective compounds had been developed over the years to reduce the levels of radiation-induced free radicals within the cell (Stone *et al*. 2004).

Ginseng is a natural product with worldwide distribution, and many reports have shown that it had a significant antineoplastic (Chang *et al*. 2003) and other pharmacological activities (Kitts & Hu 2000). Ginseng was found to increase the number of bone marrow cells, spleen cells, granulocyte-macrophage colony-forming cells, and circulating neutrophils, lymphocytes and platelets in irradiated mice. In addition, ginseng induced the endogenous production of cytokines such as Interleukin (IL)-1, IL-6, Interferon (IFN)-γ and IL-12, which were required for hematopoietic recovery, and was able to enhance T cell helper1 (Th1) function while interfering with the Th2 response in irradiated mice (Song *et al*. 2003). These findings indicate that ginseng may be a useful agent to reduce the time necessary for reconstituting hematopoietic cells after irradiation.

Ginseng and its partially purified constituents had potential radioprotective properties (Yun *et al*. 2001; Kim *et al*. 2003; Lee *et al*. 2004). It appears to be a promising radioprotector for therapeutic or preventive protocols capable of attenuating the deleterious effects of radiation on human normal tissue, especially for cancer patients undergoing RT (Jagetia & Baliga 2002). However, reports on the radioprotective effects of ginseng, had primarily been done in non-human models.

Ionizing radiations induced chromosomal aberrations that may manifested as breaks and fragments, which appears as micronuclei (MN) in the rapidly proliferating cells. The formation of MN in cytokinesis-blocked peripheral blood lymphocytes was one of the most sensitive biomarkers for

*Corresponding author. Phone: +62-21-7513906,
Fax: +62-21-7657950, E-mail: k_lusiyanti@batan.go.id

assessing the effectiveness of many chemicals to encounter genotoxicity or radiation damage *in situ* (Senthamizhchelvan *et al*. 2009; Fenech *et al*. 2003). MNs have also been used extensively in studies as an easily evaluated indicator of deoxyribonucleic acid (DNA) damage. Study provided evidence of how analyses among genetic end points in the cytokinesis-block MN assay could provide information concerning abnormalities of cell division and possibly about structural chromosomal rearrangements induced by clastogens (IAEA 2001).

The aim of this study was to evaluate the radioprotective potential of ginseng against genotoxicity of gamma irradiation manifested as MN induced in cultured blood lymphocytes.

MATERIALS AND METHODS

Ginseng Extraction. Korean red ginseng extract was purified from *Panax ginseng* at Korea Institute of Radiological and Medical Sciences (KIRAMS) with procedures according to Ahn *et al*. (2006) and Song *et al*. (2003). In brief, fresh roots of *P. ginseng* that had grown for 6 years were washed, steamed at 100 ^{o}C for 2-3 h, and dried. The dried red ginseng roots were boiled in 4-5 volumes of water for 3 h, and the supernatants were concentrated. The concentrated extract was dissolved in phosphate buffer saline (pH 7.4). *P. ginseng* is the saponin glycosides (ginsenosides) of which there are some majors and other small amount constituents.

Irradiation. Two milliliters of peripheral blood samples were collected in sterile heparinised vacutainers (Becton Dickinson) from two healthy volunteers (all males) aged of 42 and 47 years old. Each sample were irradiated with gamma rays at doses of 0.0, 0.5, and 1.0 Gy and at a dose rate of 3.16 Gy/min in ^{137}Cs Gamma-cell 3000 Elam Nordion International machine located in KIRAMS.

Lymphocytes Culture. After irradiation, blood samples were put into culture solution containing 8.5 mL of RPMI 1640 medium with L-glutamine and 25 mM HEPES buffer (Gibco Laboratories) supplemented with 10% fetal calf serum (Gibco) and antibiotics. Ginseng was added directly at concentrations of 0, 100, and 1000 µg/mL working doses. Purified phytohaemagglutinin (30 µg/mL; Sigma) was added as mitogen. Treated and control (without ginseng treatment) bloods were cultured at 37 ^{o}C for 48 h in a humidified atmosphere containing 5% CO_2. MN yield was determined with cytokinesis-blocked (CB) assay. After an incubation period of 72 h the cells were collected, treated with a hypotonic solution of 0.075 M KCl (cooled at 4 ^{o}C) and prefixed with 2 mL of cold pre-fixative solution (3% formaldehyde in fresh fixative solution). Fixative was done with a mixture of methanol/glacial acetic acid. After fixation the cells were dropped onto clean slides and allowed to dry. After mounted, MN was scored according to IAEA (2001). For 24 h treatment before irradiation, blood was mixed with culture medium in conical tube and kept in incubator at 37 ^{o}C for 24 h and then irradiated, cultured and harvested for MN as above.

Micronuclei Scoring. Frequencies of MN was conducted under a Nikon microscope with 100x magnification. The MN frequencies were scored according to the criteria proposed by Fenech *et al*. (2003) and IAEA (2001) as follow: the diameter of an MN was less than one third of the diameter of the MN, it is non-refractile and is not linked to the macronucleus by a nucleoplasmic bridge. MN partly overlapping with the nucleus or with each other was also taken into account. One thousand binucleated (BN) cells were scored on one or two slides per individual.

Statistical Analysis. The results obtained from all the groups were expressed as mean. Anova test was used to find out whether mean of sample drawn from various groups deviates significantly. The significance of the results was computed at the levels of $P < 0.05$.

RESULTS

Induction of MN After Irradiation. This study showed that the yields of MN in control pheripheral blood lymphocytes (PBL) (without ginseng treatment) were consistently higher for higher doses of radiation. Radiation clearly increased the MN yield in PBL that was in a dose-dependent manner. Irradiation with gamma rays at doses of 0, 0.5, and 1.0 Gy without the presence of ginseng extract yielded MN frequency of 0.008, 0.054, and 0.281, respectively, per 1000 binucleated (BN) cells. MNs appeared as rounded shape next to the BNC within a cytoplasm that painted lighter than the body of BNC and MN (Figure 1).

Ginseng Potential on MN Induction. The addition of ginseng (100-1000 µg/mL) did not reduce or suppress the MN yields eventhough it was in higher concentration (Figure 2). This result was unexpected since many studies reported that ginseng act as a radioprotector. Treatment with ginseng for 24 h before radiation exposure results in no suppression in MN yields for all ginseng concentrations tested. It means that pre-treated ginseng extract appeared to have no radioprotective on MN (Table 1). In general,