# TOXICITY OF A TRADITIONAL CHINESE MEDICINE, *GANODERMA LUCIDUM*, IN CHILDREN WITH CANCER

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## ABSTRACT

### Background
Cancer is one of the most common severe diseases in Canadian children, and chemotherapy treatment leads to numerous, potentially fatal, adverse side effects including febrile neutropenia and leukopenia. In an attempt to prevent opportunistic infections, *Ganoderma lucidum*, a mushroom that has been used in Traditional Chinese Medicine for thousands of years, is being used by some people as an adjunctive to chemotherapy to help boost the immune system. Although extensive research is being conducted to determine its immunostimulatory properties, there is essentially no data on toxicity.

### Objectives and Methods
The purpose of this study was to determine toxicity of low and high concentrations of 3 different extracts of *G. lucidum* (GL, Reishi and PSGL) on the viability of 1) Jurkat E6.1 cells, 2) LG2 cells, and 3) PBMCs isolated from a) healthy adults, b) healthy children, and c) paediatric patients undergoing chemotherapy.

### Results
When Jurkat E6.1 and LG2 cells were treated with increasing concentrations of the 3 extracts, both time- and concentration- dependent decreases in cell viability were observed. However, when human PBMCs were treated with the same extracts, variable results were obtained. Although there was no consistent pattern, toxicity was observed in PBMCs.

### Conclusion
This is the first study that examines the toxicity of 3 different extracts of *G. lucidum* in both adults' and children's PBMCs. Contrary to previous belief, our results suggest that extracts of *G. lucidum* should be used with caution as there appears to be potential for toxicity.

**Keywords**: *Ganoderma lucidum; Ling-zhi; Reishi, chemotherapy; paediatric cancer; immunotoxicity*

Among children in Canada, cancer is one of the most common severe diseases. Cancer rates are the highest within the first few years of life, affecting approximately one in 10 000 children annually.[1] The most common form of cancer in paediatric patients is acute lymphoblastic leukaemia, which accounts for 26 % of childhood cancer.[1] Currently, the basis of therapy for most childhood malignancies is chemotherapy, as well as surgery for solid tumors. Success of chemotherapy, however, is limited by both drug resistance and dose-limiting toxicities[2]. Chemotherapy decreases immune defense, notably, via bone marrow suppression. Aggressive supportive care, including the prevention and management of opportunistic infections, is also necessary in paediatric cancer care to deal with complications such as febrile neutropenia and leukopenia.[3] Novel therapies that can potentially reduce the risk of infections would be of great

benefit to paediatric oncology patients undergoing chemotherapy.

Currently, the relatively high rate of adverse side effects of chemotherapy and the lack of novel treatments to treat these side effects has increased interest in approaches using natural medicines or complementary and alternative medicines (CAM). As more and more patients are using CAM as adjunctives to chemotherapy, it is important to address many issues people using CAM do not consider, such as interactions and toxicity. The use of CAM in Canada is common; in fact, CAM is offered in 18 % of paediatric institutions and 94 % of communities.[4] One of the most commonly used natural-based therapy that has emerged from Traditional Chinese Medicine (TCM) is a medicinal mushroom, *Ganoderma lucidum.* More than 30 % of paediatric cancer patients in Asian communities use *Ganoderma lucidum* as an adjunctive to chemotherapy.[5]

*Ganoderma lucidum (G. lucidum),* also known as Reishi or Ling-zhi (literally, "spiritual mushroom")[6], has been in use in TCM for over 4000 years for the general promotion of health and longevity.[7] It is a basidiomycetous, wood-decomposing fungus found on fallen trees and logs in Southeast Asia. The use of *G. lucidum* in TCM has become extremely extensive; it is used to treat disorders of many systems including reproductive, excretory, digestive, circulatory, immune, cardiovascular, nervous, muscular, skeletal, endocrine, respiratory, and the skin.[8] Contrary to TCM's holistic approach, researchers in Japan, Taiwan, China, the United States, Canada, and Poland have been studying *G. lucidum* for over 30 years in an attempt to isolate specific parts of the mushroom depending on what type of disorder one is studying.[6] The main medicinal parts of the fungus are believed to be the spores, which contain the sporophores, the fruiting body, and the mycelium. Depending on which component is used, different chemical constituents and active ingredients are present.[8] Furthermore, depending on the type of extraction method that is performed, the presence and amounts of chemical compounds vary. The two main active chemical classes from *G. lucidum* are triterpenes, which possess numerous properties, and polysaccharides, which possess immunostimulatory and antitumor properties.[8] More specifically, the hot water polysaccharide extracts isolated from the fruiting body of *G. lucidum* produce the highest percentage of biologically active ingredients for immunostimulation.[9] Multiple studies using the mononuclear phagocyte system, dendritic cells (DCs), natural killer cells (NK cells), T cells and B cells have been conducted in an attempt to demonstrate the immunostimulatory effects of *G. lucidum* and its extracts. However, little work has been done to investigate potential toxicity. Based on previous literature, extracts of *G. lucidum* may have the potential to stimulate cells of the immune system; however, immune cells are often susceptible to toxicity when treated with higher doses of substances that are stimulatory in lower concentrations.

The purpose of this study was to determine the effects of low and high concentrations of 3 different extracts of *G. lucidum* (GL, Reishi and PSGL) on the viability of 1) Jurkat E6.1 cells, 2) LG2 cells, and 3) peripheral blood mononuclear cells (PBMCs) isolated from a) healthy adults, b) healthy children, and c) paediatric patients undergoing chemotherapy. We hypothesize that at low concentrations (1 – 30 µg/mL), extracts of *G. lucidum* will stimulate cells of the immune system, and at high concentrations (200 – 350 µg/mL), extracts of *G. lucidum* will cause toxicity in cells of the immune system.

## METHODS

Dr. Arthur Weiss, University of California, kindly provided the Jurkat E6.1 cell line. This is a T lymphoblast cell line. The LG2 cell line was kindly provided by Dr. Joaquim Madrenas, Robarts Research Institute. The LG2 line, an Epstein-Barr virus-transformed cell line, is a human B lymphoblast cell line derived from a lymph node metastasis. These cell lines are maintained in cell culture media consisting of RPMI-1640 (Gibco, Mississauga, ON) supplemented with 10 % heat-inactivated fetal bovine serum (FBS) (Hyclone, Logan, UT, USA), 100 U/mL of penicillin G and streptomycin (P/S) (Gibco, Mississauga, ON), and 1 % L-glutamine (Gibco, Mississauga, ON) in a humidified chamber at 37°C and 5 % $CO_2$.

Whole blood was obtained from 3 different subject groups: 5 healthy adults, 5 healthy children ranging in age between 8 and 18 years, and 6

paediatric patients undergoing chemotherapy. Blood from the paediatric patients undergoing chemotherapy was obtained (ages ranging between 13 and 18 years). All six oncology patients, 5 males and 1 female, had acute lymphoblastic leukemia. Additionally, although they were at different stages of treatment, all six were receiving the same chemotherapy regimen: oral 6-mercaptopurine daily, methotrexate weekly, and vincristine weekly for 4 weeks, and intrathecal methotrexate every 12 weeks. Ethics approval was obtained for each patient population, and healthy children and oncology patients were recruited from the Children's Hospital of Western Ontario.

Density gradient centrifugation of whole blood in the presence of a Ficoll–Histopaque gradient was used to isolate PBMCs.[10] Approximately 30 mL of heparinized whole blood was layered on 15 mL Histopaque-1077 (Sigma-Aldrich, Oakville, ON) in a 50 mL Falcon tube (Becton Dickinson Falcon Labware, Franklin Lakes, NJ, USA), and centrifuged at 1500 rpm for 30 min to separate the whole blood into layers: plasma layer, PBMC layer, histopaque layer and red blood cell layer.[10] The PBMCs, located in the opaque interphase, were collected using a 10 mL pipette, and placed in a new 50 mL Falcon tube.[10] The PBMCs were maintained in cell culture media consisting of RPMI supplemented with 10 % heat-inactivated FBS, 100 U/mL of P/S, 1 % L-glutamine, and 2 mM 2-mercapto-ethanol (Sigma-Aldrich, Oakville, ON) in a humidified chamber at 37°C and 5 % $CO_2$.[10]

The cell lines used in our experiments were incubated in a humidified chamber in T-75 $cm^2$ flasks (Becton Dickinson Falcon Labware, Franklin Lakes, NJ, USA), and were passed every 3 days. Prior to experiments, cells, including PBMCs, were placed in 50 mL Falcon tubes and centrifuged at 1500 rpm using a Beckman GP centrifuge for 10 minutes to form a pellet. The supernatant was discarded and cells were washed 3 times, each time using 25 mL phosphate buffered saline solution (PBS) (1.15% $K_2HPO_4$, 0.2% $KH_2PO_4$, 8.0% NaCl, 0.2% KCl, pH 7.4), and were re-suspended in 10 mL of the appropriate cell culture media.

Once the cells were washed and re-suspended in cell culture media, a trypan blue exclusion assay was performed. A 50 µL aliquot of trypan blue was mixed with 50 µL of the cell suspension, and viable cells were counted using a hemacytometer (Hausser Scientific, USA). For accuracy, two corners were used and the average was taken. The appropriate cell concentration was calculated and the cells were plated.

Three different extracts were used in conducting our experiments to determine if these extracts will cause similar trends within the same cell types. The extracts used were: 1) a crude extract of *G. lucidum* (GL) kindly donated by Jeff Chilton from Nammex Inc. (Vancouver, BC), 2) a polysaccharide extract of *G. lucidum* (PSGL) from Dr. Ricky Man (Hong Kong University, Hong Kong), and 3) a commercially available extract of *G. lucidum* (Reishi) in capsule form purchased from a Chinese Herbal Supplement Supermarket (Eternal Trading Inc., Markham, ON). All three extracts were stored in a cool, dry and dark environment, and were dissolved in the appropriate cell culture media when needed. To eliminate batch-to-batch variations, and to control for the lack of a standardized product, the same batch of each extract was used to conduct all of the experiments. Furthermore, preliminary dose-dependent assays were conducted to determine the appropriate doses of the extracts to be used in this study, and to determine if the different extracts exhibited similar properties.

Once cells were washed and the trypan blue exclusion assay was performed, the appropriate cell concentration was calculated to perform a validated toxicity assay.[11] In flat-bottom 96 well plates (Becton Dickinson Falcon Labware, Franklin Lakes, NJ, USA), Jurkat E6.1 cells, and LG2 cells were plated at 500 000 cells/mL or 25 000 cells per well; PBMCs were plated at 1 million cells/mL or 50 000 cells per well. For each assay, standard curves were constructed using 25, 50 and 100 % of cell concentrations. Furthermore, controls and blanks were used consisting of the appropriate cell culture media to ensure the reliability and validity of the assays. Extracts were dissolved in the cell culture media particular to the cell type being used. Cells were incubated with both lower concentrations of extracts, ranging from 1 µg/mL to 50 µg/mL, to determine immunostimulatory effects, and higher concentrations of extracts, ranging from 50 µg/mL to 350 µg/mL, to determine toxic effects. After the cells and extracts were plated, plates were incubated for 24 hours or 48 hours in a humidified

chamber at 37°C and 5 % $CO_2$. After incubation, 25 µL of 5 mg/mL of MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) (Sigma-Aldrich, Oakville, ON) dissolved in HEPES buffer solution (40% HEPES powder, 2% $NaHCO_3$, 7.3% NaCl, 0.45% KCl, 2.95% $MgSO_4 \cdot 7H_2O$, 0.147% $CaCl_2 \cdot H_2O$, 1.8% glucose, pH 7.4) was added to each well, and 4 hours later 100 µL of stop solution (consisting of 50% N, N-dimethylformamide (BDH Inc., Toronto, ON) and 20% sodium dodecyl sulphate (Sigma-Aldrich, Oakville, ON) in $ddH_2O$) was added.[11] Plates were stored overnight at room temperature in the dark.[11] The next morning, absorbance was measured using a Molecular Device Spectrophotometer (Beckman, Palo Alto, CA, USA) at a wavelength of 590 nm.[11] Data analysis was performed using Softmax™ Molecular Devices Group Analytical Software version 2.35.

Assays were performed in quadruplicate. The data are represented as the mean ± SEM of 4-6 separate experiments. Statistical analysis was performed using GraphPad InStat. Raw data were analyzed using a One-way Analysis of Variance (ANOVA) with post hoc Dunnett's Multiple Comparison test. Statistical significance was assessed at $p<0.05$ as compared to control.

## RESULTS

To ensure the validity of our assays, cell viability of controls was measured for each individual experiment. Cell viability did not fall below 92 % viability for all experiments and cell types, and ranged between 92.64 – 103.62 %.

No significant increases in cell viability were observed after treatment of Jurkat E6.1 cells with GL, Reishi or PSGL (figures 1A and 1B); however, significant decreases in cell viability were observed. Although no significant decreases were observed after 24 hr Reishi treatment, significant decreases in cell viability occurred starting at 20 µg/mL Reishi (70.0 % ± 6.9) after 48 hr treatment (figure 1A). After treatment with GL, significant decreases in cell viability were only observed at higher concentrations after both 24 hr (<59.7 % ± 5.6) and 48 hr treatment (<68.2 % ± 3.7) (figure 1B). Decreases in cell viability were observed after treatment with lower (<83.8 % ± 1.0) (figure 1A) and higher concentrations (<74.0 % ± 4.6) of PSGL (figure 1B) at both time points.

**FIG. 1 A**

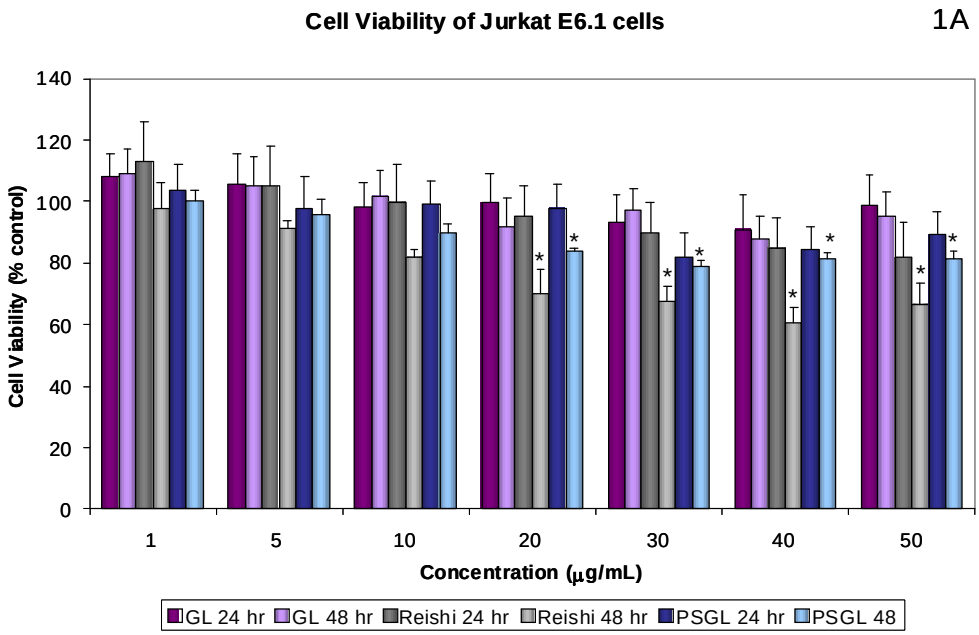


**FIG. 1B**

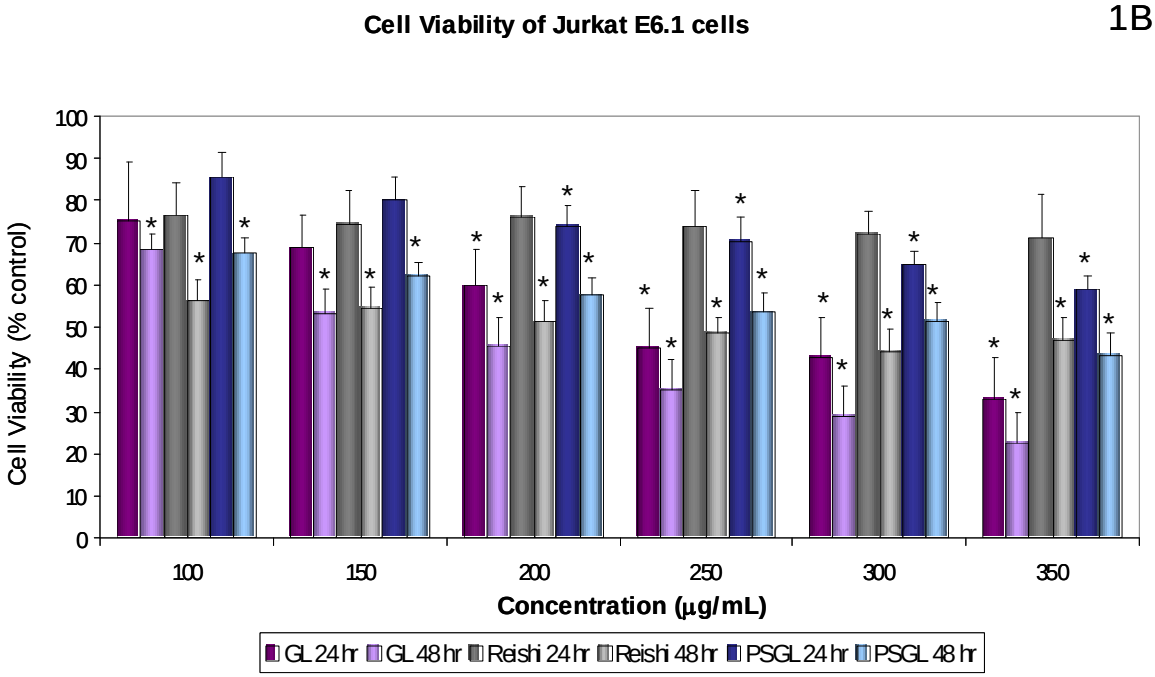



**FIG. 2A**

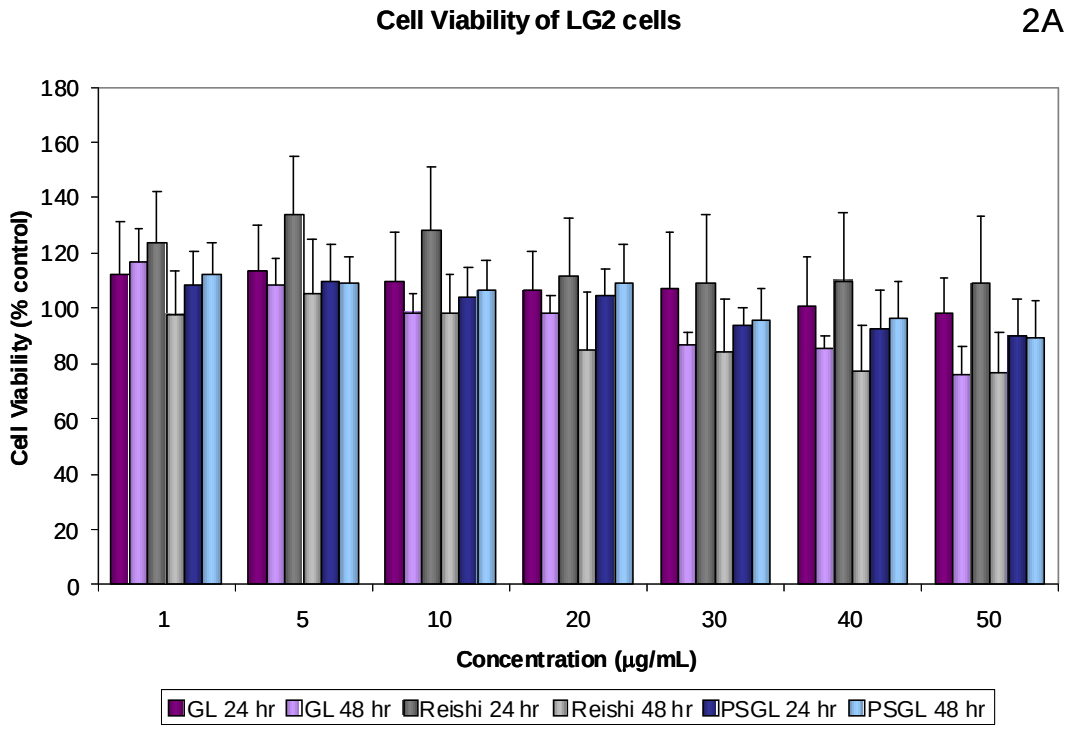
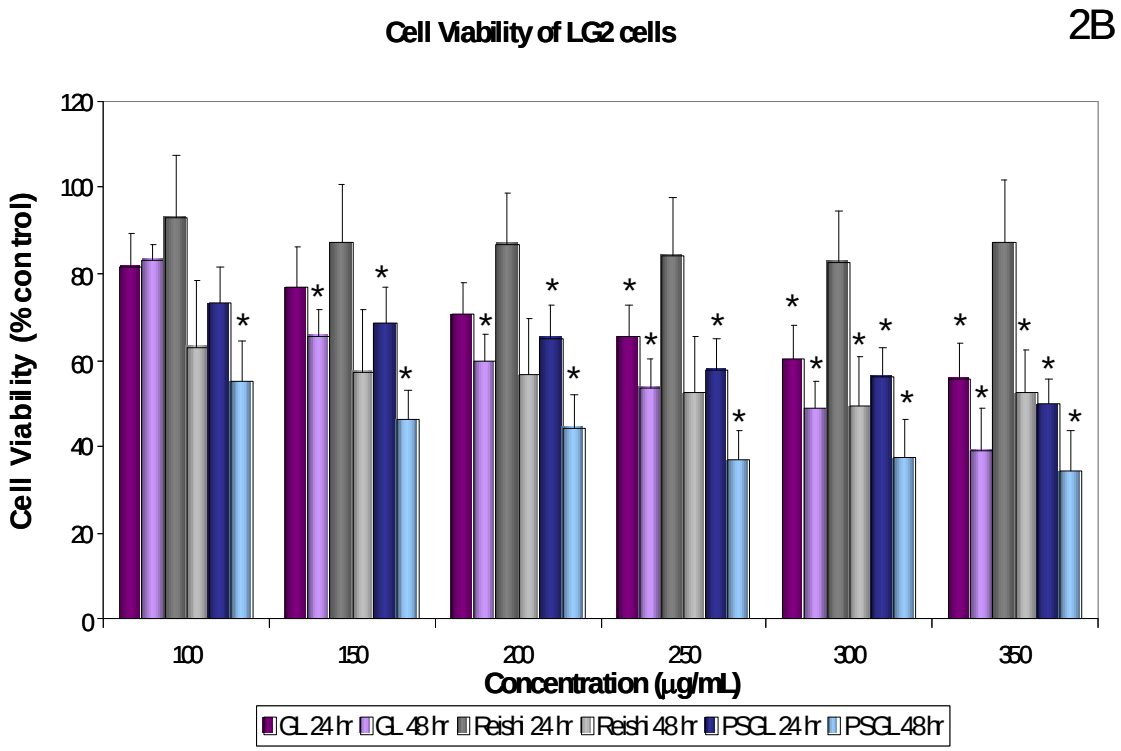


**FIG. 2B**




At low concentrations of all three extracts, no significant increases or decreases were observed in LG2 cells (figure 2A). In contrast, at high concentrations, significant decreases in LG2 cell viability as compared to control were noted (figure 2B). GL and PSGL caused significant decreases in cell viability starting at 24 hr (<65.4 % ± 7.7 and <68.3 % ± 7.6, respectively) and 48 hr treatment (<65.5 % ± 6.6 and <60.1 % ± 9.8, respectively) (figure 2B). Reishi did not cause significant decreases in cell viability after 24 hr treatment; however, after 48 hr treatment, significant decreases in cell viability were observed (<49.2 % ± 10.0) (figure 2B).

After 24 hr treatment at low concentrations (figure 3A), GL and PSGL did not cause significant increases in cell viability of PBMCs from healthy adults; however, with the use of Reishi there was a significant increase in cell viability at 1 µg/mL as compared to control (181.6 % ± 25.3). A significant increase in cell viability was also observed after 48 hr treatment of 5 µg/mL GL (202.9 % ± 32.4), and 1 and 5 µg/mL PSGL (143.6 % ± 16.3 and 145.4 % ± 17.8, respectively) (figure 3A).

However, as concentrations increased considerably, significant decreases in cell viability occurred after 24 hr treatment of GL (74.3 % ± 5.3) and PSGL (54.8 % ± 6.7) (figure 3B). Interestingly though, significant increases in cell viability were observed after 300 µg/mL Reishi treatment after 24 hrs (237.9 % ± 29.2) (figure 3B). After 48 hr treatment with Reishi, significant increases in cell viability (>183.7 % ± 26.9) were still observed at 100, 300 and 350 µg/mL (figure 3B).

**FIG. 3A**

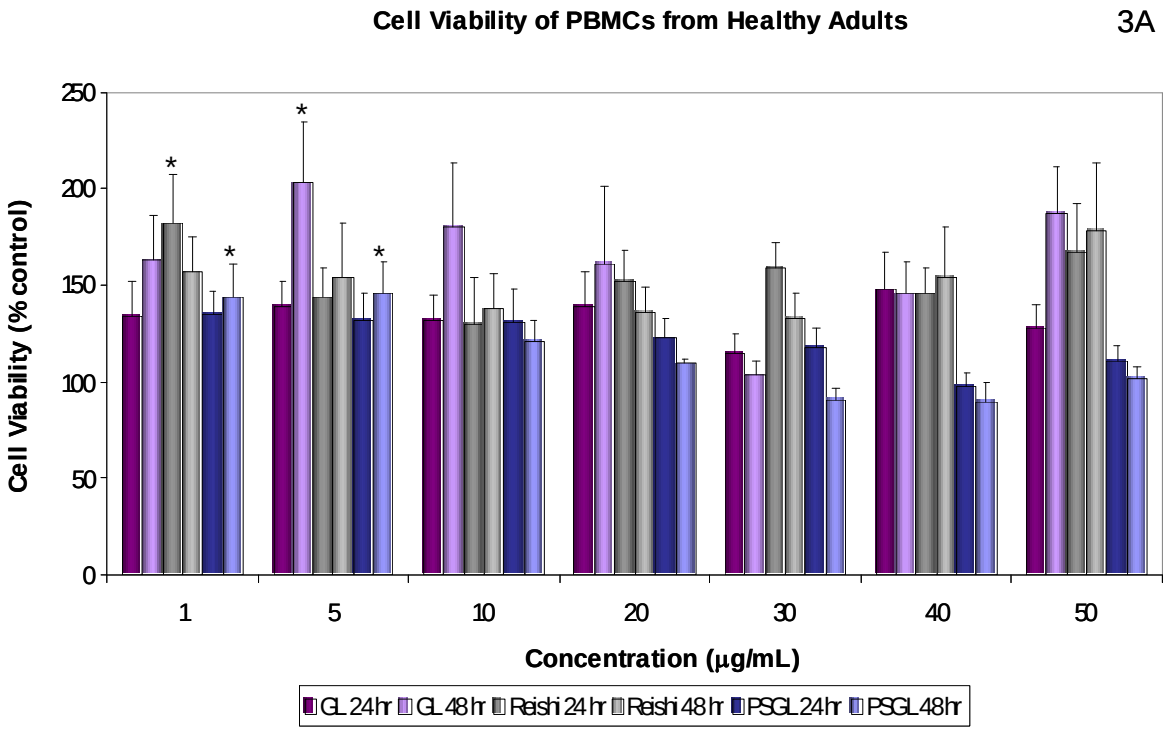



**FIG. 3B**

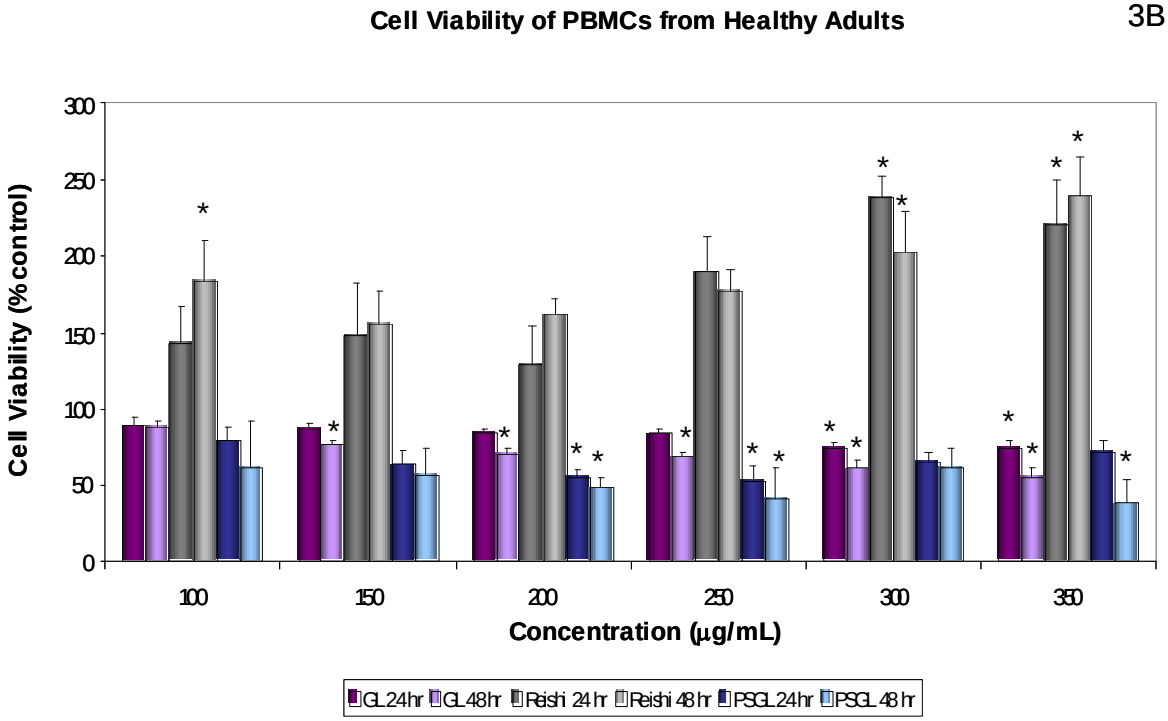
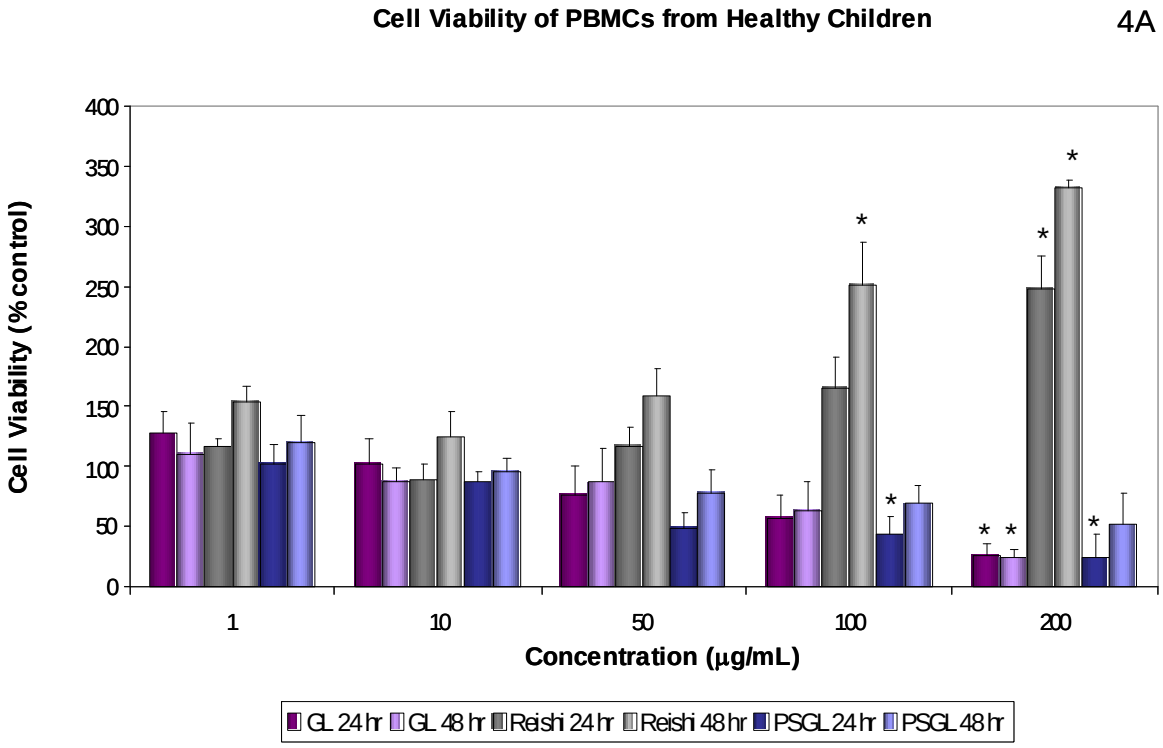
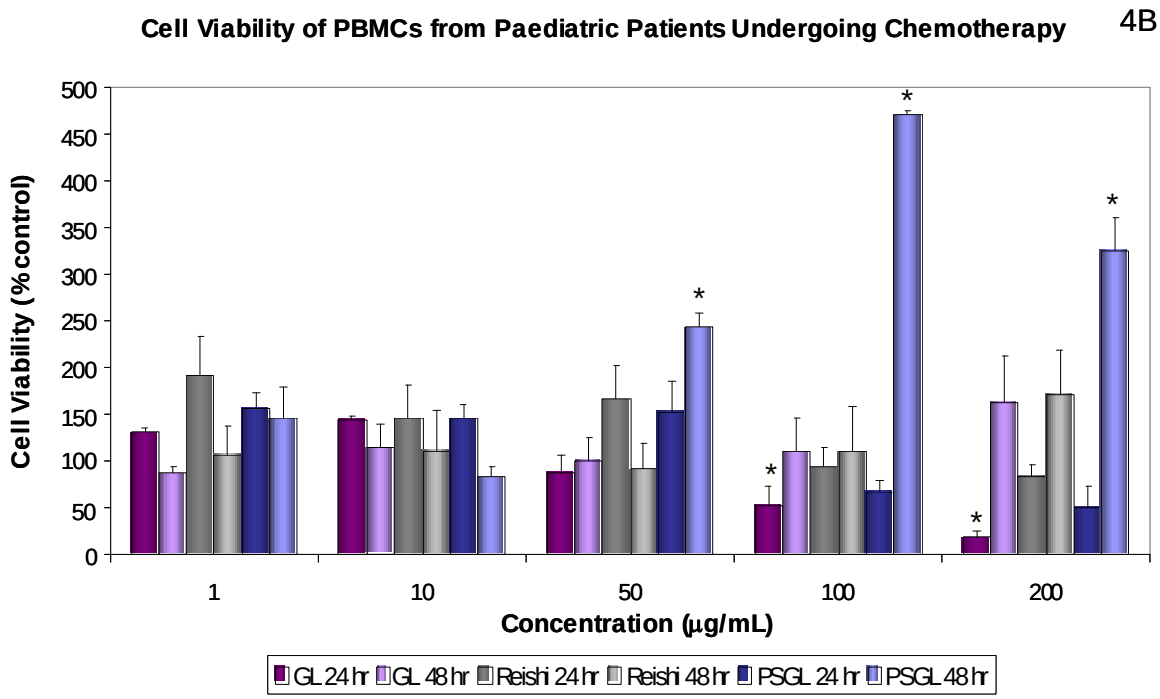


**FIG. 4A and FIG. 4B**



PBMCs isolated from the whole blood from healthy children were incubated with GL, Reishi and PSGL at 1, 10, 50, 100 and 200 µg/mL for 24 and 48 hrs. At 24 and 48 hrs, there were concentration-dependent decreases in cell viability after GL and PSGL treatment (figure 4A). Treatment with Reishi, however, appeared to have the opposite effect: as concentration increased, there was an increase in cell viability with significant increases occurring at 100 (>251.4 % ± 18.3) and 200 µg/mL (>247.5 % ± 15.8) after 24 and 48 hr treatment (figure 4A).

PBMCs were also isolated from paediatric patients undergoing chemotherapy, and were treated with GL, Reishi and PSGL at 1, 10, 50, 100 and 200 µg/mL for 24 and 48 hrs. At 24 hr treatment, concentration-dependent decreases in cell viability were observed with significant decreases in cell viability occurring at 100 (52.1 % ± 20.3) and 200 µg/mL (18.1 % ± 6.8) GL (figure 4B). Although slight increases were observed at lower concentrations of Reishi and PSGL after 24 hrs, these increases were not found to be significant (figure 4B). After 48 hr treatment, GL and Reishi did not cause any significant changes in cell viability; however, PSGL caused significant increases in cell viability at higher concentrations (> 243.3 % ± 16.1) (figure 4B).

## DISCUSSION

The use of CAM by patients with cancer is common and likely to increase. Typically, CAM is used to alleviate side effects and to enhance well-being; additionally, it may also be used as therapy for malignancy. However, there has been very little study of the use of CAM in childhood cancer and almost no work on potential toxicity.

In the present study, variable increases in cell viability occurred after treatment with extracts from *G. lucidum*; however, as aforementioned, immune cells are often susceptible to toxicity when treated with higher doses of substances that are stimulatory in lower concentrations. Consequently, there were consistent significant decreases in cell viability indicating that *G. lucidum* can produce toxicity in cells of the immune system. We found concentration- and time-dependent decreases in cell viability when T cells are treated with extracts of *G. lucidum.* Contrary to previous research[12-14], significant decreases in T cell viability were observed at higher concentrations, and these findings were consistent among all 3 extracts of *G. lucidum* used in this study. This significant decrease suggests that, contrary to popular belief[15], extracts from *G. lucidum* are indeed toxic to T cells. Thus, prolonged use of *G. lucidum* at higher concentrations will result in greater toxicity of T cells. These findings are in contrast to previous research: mice splenocytes co-incubated with ConA and four different polysaccharide extracts of *G. lucidum* (PL-1 – PL-4) ranging between 1 - 100 µg/mL for 44 hrs demonstrated a significant concentration-dependent increase in T cell proliferation as assayed using the MTT method.[12] Although these researchers used the same assay as the present study to measure changes in T cell numbers, differences in the results obtained may be a result of different cell types used, or due to differences between extracts.

The present study indicates that there are concentration- and time-dependent decreases in cell viability when B cells are treated with extracts of *G. lucidum*. Again, contrary to previous research[16-18], significant decreases in B cell viability were observed at higher concentrations, and these findings were consistent among all 3 extracts of *G. lucidum* used in this study. This significant decrease in cell viability indicates that B cell toxicity is a consequence of treatment with extracts of *G. lucidum*; this finding is in contrast with previous research that claimed no toxicity results from the use of *G. lucidum* extracts.[15] Again, prolonged use of *G. lucidum* extracts at higher concentrations will result in greater toxicity of B cells. These findings are in contrast to previous research in which a polysaccharide extract of *G. lucidum* was found to stimulate the activation, differentiation and proliferation of B lymphocytes.[18,19] Although these researchers used a MTT assay similar to the present study to measure changes in B cell numbers, again, differences in the results obtained may have arisen from the use of different cell types or variation between extracts.

PBMCs are comprised of a mixture of monocytes and lymphocytes, which are essentially blood leukocytes, from which granulocytes have been separated and removed. Specific percentages of T helper cells, T cytotoxic cells, B cells and

NK cells in peripheral blood are 55 %, 25 %, 10 % and 10 %, respectively. Furthermore, as previously mentioned, these various cell types release numerous signal factors and modulators, which, in turn, exert other effects on multiple components of the immune system. Not only do PBMCs contain numerous discrete cellular sub-populations, there are age differences that need to be taken into account as well.

The present study indicates that there are not significant concentration-dependent decreases in either cell viability or cell proliferation when PBMCs are treated with extracts of *G. lucidum* ranging from 1 – 50 µg/mL. Instead, to some extent, increases in cell viability and cell proliferation occurred after 24 hr and 48 hr treatment when PBMCs from healthy adults were treated with GL, Reishi or PSGL at the lower concentrations. In contrast, no significant increases in either viability or proliferation occurred when PBMCs from either healthy children or paediatric oncology patients were treated with lower doses of extracts for 24 and 48 hrs. At higher doses (≥ 100 µg/mL), concentration-dependent decreases in cell viability were observed after PBMCs from all test groups were treated with either GL or PSGL for 24 and 48 hrs with the exception of 50, 100 and 200 µg/mL PSGL treatment for 48 hrs in paediatric oncology PBMCs. On the other hand, with the Reishi extract, decreases in the cell viability of PBMCs were not as evident compared to the other extracts. Perhaps because it is a commercially available extract, additional compounds may be present to enhance its immunostimulatory properties or decrease its toxicity. Nonetheless, the addition of adulterants and the lack of a standardized extract is a concern, despite possible immunostimulatory properties. Additionally, we observed that, after treatment with *G. lucidum*, toxicity is observed in PBMCs; suggesting, that use of extracts from this fungus may not be without side effects.

Interestingly, although toxicity was observed at higher concentrations, significant increases in cell viability were also observed. These increases are similar to those observed in previous studies. For instance, in a study using PBMCs from advanced colorectal cancer patients, treatment with a polysaccharide extract of *G. lucidum* at 0.05 – 1 µg/mL resulted in increases in the cell proliferation of 56 % of patients, and decreases in 44 % of patients[14], demonstrating the importance of inter-individual variations. Furthermore, the use of Ganopoly™ in the PBMCs of advanced cancer patients resulted in significant increases in NK cells and slight increases in T cell numbers.[2] In another study, the use of two different extracts from *G. lucidum* (1 – 1000 µg/mL) had opposite effects: the extract derived from the fruiting body significantly induced proliferation of PBMCs and monocytes; whereas, the extract derived from the spore suppressed PBMC proliferation.[5] These previous studies illustrate the differences that can be obtained with the use of different extracts in PBMCs, consistent with findings in the present study. Because of the lack of a standardized product, and therefore, different active components and their levels present, inconsistencies between studies are likely and could be a critical factor in explaining the discrepancies observed in this study compared with other studies. Furthermore, the age-related discrepancies that were observed could be explained by a couple of key factors. Firstly, age-related differences in lymphocyte subsets may account for the different results obtained between the groups, and secondly, paediatric patients undergoing chemotherapy will have altered populations of PBMCs due to the immunosuppressive and toxic effects of chemotherapy.

In the present study, PBMCs were used in addition to cell lines, as the use of PBMCs as a predictive model of toxicity has been used in a number of studies. Also in the present study, variable increases in cell viability occurred after treatment with extracts from *G. lucidum*. Based on these results, we believe that it cannot be concluded with certainty that *G. lucidum* causes immunostimulation. Given that there were consistent significant decreases in cell viability demonstrated, this suggests that *G. lucidum* does cause toxicity in cells of the immune system. Therefore, if patients are willing to use *G. lucidum* as an adjunctive to chemotherapy at lower concentrations, caution should be used, as the potential for toxicity does appear to exist. Additionally, further research is required to verify plasma concentrations after oral use of *G. lucidum* to determine whether high toxic levels are reached. Further studies are also required to elucidate the mechanisms for this potential

toxicity. As well, caution needs to be exercised when planning studies of the use of *G. lucidum* or its extracts in the therapy of patients with cancer.

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**REFERENCES**

1. Gao RN, Levy IG, Woods WG, Coombs BA, Gaudette LA, Hill GB. Incidence and mortality of neuroblastoma in Canada compared with other childhood cancers. Cancer Causes Control 1997;8:745-754.
2. Gao Y, Zhou S, Jiang W, Huang M, Dai X. Effects of Ganopoly™ (A *Ganoderma lucidum*Polysaccharide Extract) on the Immune Functions in Advanced-Stage Cancer Patients. Immunol Invest 2003;32:201-215.
3. Phillips E. Hotspot: Advances and Challenges in the Management of Febrile Neutropenia. Rapid Response Radiotherapy Program 2001;4:1-2.
4. Johnston D, Nagel K, O'Halloran C, et al. Complementary and alternative medicine in pediatric oncology: availability and institutional policies in Canada – A report from the children's oncology group. Pediatr Blood Cancer 2006;47:955-958.
5. Chan W, Lam D, Law H, et al. Ganoderma lucidum mycelium and spore extracts as natural adjuvants for immunotherapy. J Complem Altern Med 2005;6:1047-1057.
6. Chen J, Chen T. Chinese Medical Herbology and Pharmacology. England: Art of Medicine Press, 2003.
7. Sliva D. *Ganoderma lucidum* (Reishi) in Cancer Treatment. Integr Cancer Ther 2003;2:358-364.
8. Shiao M. Natural products of the medicinal fungus *Ganoderma lucidum*: Occurrence, biological activities, and pharmacological functions. Chem Rec 2003;3:72-180.
9. Zhang H, Lin Z. Hypoglycemic effect of *Ganoderma lucidum* polysaccharides. Acta Pharmacol Sin 2004;2:191-195.
10. Mossman T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity. J Immunol Methods 1983;65:55-63.
11. Bach M, Brashler J. Isolation of subpopulations of lymphocytic cells by the use of isotonically balanced solutions of Ficoll. I. Development of methods and demonstration of the existence of a large but finite number of subpopulations. Exp Cell Res 1970;61;387-396.
12. Bao XF, Wang XS, Dong Q, Fang JN, Li XY. Structural features of immunologically active polysaccharides from *Ganoderma lucidium*. Phytochemistry 2002;59:175-181.
13. Wang Y, Khoo K, Chen S, Lin C, Wong C, Lin C. Studies on the Immuno-modulating and anti-tumor activities of *Ganoderma lucidum* (Reishi) polysaccharides: functional and proteomic analyses of a fucose-containing glycoprotein fraction responsible for the activities. Bioorg Med Chem 2002;10:1057-1062.
14. Chen H, Tsai Y, Lin S, et al. Studies on the Immunomodulating and antitumor activities of *Ganoderma lucidum* (Reishi) polysaccharides. Bioorg Med Chem 2004; 12:5595-5601.
15. Chien C, Cheng J, Chang W, et al. Polysaccharides of *Ganoderma lucidum* alter cell immunophenotypic expression and enhance CD56+ NK-cell cytotoxicity in cord blood. Bioorg Med Chem 2004; 12:5603-5609.
16. Bao X, Fang J, Li X. Structural characterization and immunomodulating activity of a complex glucan from spores of *Ganoderma lucidum*. Biosci Biotechnol Biochem 2001;65:2384-2391.
17. Shao B, Dai H, Xu W, Lin Z, Gao X. Immune receptors for polysaccharides from *Ganoderma lucidum*. Biochem Biophys Res Commun 2004;323:133-141.
18. Lin Z, Zhang H. Anti-tumor and immunoregulatory activities of *Ganoderma lucidum* and its possible mechanisms. Acta Pharmacol Sin 2004;11:1387-1395.
19. Zhang J, Tang Q, Zimmerman-Kordmann M, Reutter W, Fan H. Activation of B lymphocytes by GLIS, a bioactive proteoglycan from *Ganoderma lucidum*. Life Sci 2002;71:623-638.