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Immunomodulatory efficacy and safety of *Ganoderma lucidum* broken spore supplement in patients after chemotherapy

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ABSTRACT

The immune system requires a long time to recover following chemotherapy completion. *Ganoderma lucidum* broken spores (GLBS) have a strong immunomodulatory effect. This study aimed to explore the immunomodulatory efficacy, quality of life, and safety of GLBS in patients after the chemotherapy completion. Forty-four participants were equally randomized to take GLBS 750 mg per day for 8 weeks or a placebo. Immunoglobulin (IgG, IgM, IgA, and IgE) levels, complete blood counts, liver functions, renal functions, and adverse events were measured at weeks 0, 4, and 8. Quality of life was assessed before and after study participation. At 8 weeks, white blood cell and absolute neutrophil count levels in the GLBS group significantly increased (p=0.031 and 0.021, respectively). However, the other immune levels in the GLBS group showed no significant difference from the placebo group. No significantly changes were observed in renal and liver functions. Mild adverse events, such as dry mouth, were found. Furthermore, the quality of life in the GLBS group tended to be better than the placebo group. According to the results, GLBS consumption tended to improve quality of life without serious adverse events and stimulates some immune cells in patients after the completion of chemotherapy.

Keywords: chemotherapy; *Ganoderma lucidum*; immunoglobulin; quality of life; Reishi; safety

1. INTRODUCTION

Cancer is expected to rank as the leading cause of death across the world. The World Health Organization (WHO) reported that new cases of breast, lung, and colorectal cancer are the most common cancers worldwide (WHO, 2020). Chemotherapy is the most common and well-known of the cancer treatments. One of the serious side effects of chemotherapy is its myelosuppressive effects, which lead to a decrease in white blood cells (WBCs), red blood cells, and immune cells (Katzung et al., 2019). A previous study reported that immune cells such as B cells, which produce immunoglobulin (Ig), and helper T cells had not recovered 9 months after the completion of

chemotherapy (Verma et al., 2016). Unexpected infections should therefore be of concern.

Nowadays, traditional Chinese medicine (TCM) is one of the choices for complementary and alternative medicines and is popular for supporting cancer treatment, including the reduction of side effects and the improvement of physical health (Hack et al., 2018). There are several Chinese herbal medicines that show herbal pharmacological activities. One of the famous Chinese herbs is *Ganoderma lucidum* (GL), known as 'Lingzhi' in China or 'Reishi' in Japan. It contains plenty of bioactive compounds, such as polysaccharides and triterpenes (Sanodiya et al., 2009), and is composed of a fruiting body and spores. GL spores have a higher bioactivity than other parts of the plant, with broken spores (GLBS) having more active substances than the unbroken ones (Soonthornchareonnon, 2008). The most effective GLBS polysaccharides and triterpenes were α- or β-(1→3)-, (1→6)- glucans (Benkeblia, 2015) and ganoderic acid (Wang et al., 2020), respectively. GLBS show several pharmacological effects, including immunomodulatory and antitumor effects. Previous studies have reported that GLBS have cytotoxic effects (Zhu et al., 2000) and immunomodulatory activities, as well as a stronger stimulation of tumor necrosis factor (TNF)-α, interleukin (IL)- 6, IL-2, IL-4, macrophages, and B lymphocytes than the fruiting body (Yue et al., 2008). No serious adverse events have been reported (Zhao et al., 2012). A supplement of GL polysaccharide for 8 weeks in patients with advanced stage cancer significantly increased these cytokines (Gao et al., 2003). Although GL could induce Igs in both the small intestines of rats (Kubota et al., 2018) and human peripheral B cells (Lin et al., 2006), the effect of GLBS in patients who have completed chemotherapy was suspected. There is currently no consensus on the most effective dosage of GLBS. The dose and dosage regimen vary among previous studies and commercial products. Researchers are currently focused on GLBS at 750 mg per day, which is the recommended commercial dose for healthy people in Thailand.

This study aimed to monitor serum Ig levels to evaluate the immunomodulatory efficacy and safety of GLBS in patients who completed chemotherapy treatment. Patients' quality of life was also reported.

2. MATERIALS AND METHODS

2.1 Study population

This study was conducted at the chemotherapy unit of the Police General Hospital and the National Cancer Institute, Thailand, between November 2018 and December 2019. The study protocol was approved by the Ethics Committee of the Police General Hospital (Sh230352/60) and the National Cancer Institute (EC COA 004/2019). The inclusion criteria were participants aged 18 years or older, diagnosed with stage 1-3 breast or colorectal cancer, who had completed a chemotherapy course within 1 month, a good performance status, and a life expectancy longer than 2 months. Exclusion criteria were pregnancy or breast-feeding, a history of mushroom allergy, immunodeficiency, smoking, and drinking more than 141 g of alcohol per week. Less than 80 percent compliance, adverse events of grade 3-5 (determined using the common terminology criteria for adverse events; CTCAE), or the use of other immunomodulating agents during the study led to termination from the study. Sample sizes were calculated using the G*power program: effect size = 0.25, alpha = 0.05, power = 90. The total calculated sample size was 36, divided equally between both groups.

2.2 Randomization and blinding

Eligible patients were randomized by block randomization, with a block size of 4 and a ratio of 1:1, to receive GLBS or a placebo for an 8-week treatment period. Due to the limited number of patients, no stratified sampling was undertaken according to the type and stage of cancer. Patients were assigned consecutive randomized numbers as they entered this study. Patients and physicians were blinded to the interventions during this study. The individual intervention was revealed in cases where a serious adverse event was reported.

2.3 Interventions

Participants were screened using their medical history and a detailed interview; written informed consent was given on the last day of their chemotherapy course. Participants were randomized into either the 750 mg GLBS or placebo groups, taking 1 capsule (250 mg) 3 times daily, before meals, for 8 weeks. The appearance and packaging of all products were similar. Products were provided by Z Natural Pharmaceutical Co. Ltd., Bangkok. Participants attended appointments every 4 weeks. Blood samples were obtained for the analysis of IgG, IgM, IgA, and IgE levels, complete blood counts, liver function tests, such as alanine transaminase (ALT), aspartate transaminase (AST) and alkaline phosphatase (ALP), and renal function tests, such as blood urea nitrogen (BUN) and serum creatinine, at baseline, week 4, and week 8 of the study. Serum Ig levels were determined by a commercial nephelometry assay using a BN ProSpec system. At every visit, the physician carried out a physical examination and asked if there were any adverse events. The participants noted their compliance and any adverse events themselves, using the safety sheet and calendar provided, and a reminder of the next appointments, administrations, compliance, and adverse events were given on every visit and by telephone every 2 weeks. Compliance was assessed using the patients' self-reported compliance information and pill counts. At baseline and week 8, participants answered quality of life questionnaires using the functional assessment in cancer therapy-general (FACT-G) version 4 (Cella et al., 1993). The Thai version of the FACT-G questionnaire had a reliability of 0.75-0.90 with Cronbach's alpha coefficient (Ratanatharathorn et al., 2001) and had 27 items, with item scales ranging from 0-4 (not at all - very much), divided into 4 domains: physical, emotional, social, and functional well-being. The FACT-G total scores ranged from 0-108, with higher scores corresponding to a better quality of life. The severity of adverse events was evaluated using CTCAE 5.0 grading criteria.

2.4 Statistical analyses

All data were analyzed using a per-protocol model. The significance levels were set at $p<0.05$. All statistical analyses were performed using Statistical Package for the Social Sciences (SPSS) version 22.0 (SPSS Inc., Chicago, IL, USA). The Shapiro-Wilk test was used to determine the normal distribution of variables. The characteristic data

and adverse events were described as frequency, percentages, or means, while the laboratory data were expressed as median and interquartile range. Statistic differences between various groups for demographic and laboratory data were assessed using an independent t test, Kruskal Wallis H test, χ^2 test, or Fisher's exact test, as appropriate. The quality of life was analyzed by the Mann Whitney U test, comparing both groups.

3. RESULTS

3.1 Participant characteristics

Recruitment was conducted from November 2018 through December 2019. The last participant was followed up until March 2020. Figure 1 presents a flowchart of participant recruitment. A total of 68 cancer patients were screened to determine their potential eligibility. Of these, 48 patients agreed to participate in the study and were randomly assigned to the GLBS or placebo groups. A total of 44 patients, divided equally into 22 per group, completed the 8-week study. The reasons for exclusion were unrelated product adverse events (rash after taking only 3 placebo capsules) and being lost to follow up. There were no statistically significant difference between the characteristic data of these groups (Table 1).

3.2 Immunomodulatory efficacy

3.2.1 Ig levels

At baseline, there was no significant difference among all Ig levels between groups (p>0.05). The baseline IgG levels in the GLBS and placebo group were 1,225 (468.75) and 1,260 (688.25) mg/dL, respectively. At week 4, the IgG level in the GLBS group was 1,225 (352.5) mg/dL, while the IgG level in the placebo group increased to 1,310 (522.5) mg/dL. At week 8, IgG level had increased to 1,280 (365.0) in the GLBS group and 1,415 (452.5) mg/dL in the placebo group. The baseline IgM level in the GLBS and placebo groups was 72.9 (62.57) and 76.2 (67.0) mg/dL, respectively. IgM level in the GLBS and placebo groups had decreased at week 4, to 70.1 (43.28) and 68.2 (68.18) mg/dL, respectively. At week 8, IgM level had decreased to 65.8 (42.08) mg/dL in the GLBS group, while IgM level in the placebo group had increased to 85 (66.85) mg/dL. The baseline IgA level in the GLBS and placebo groups was 276 (125.5) and 244 (166.25) mg/dL, respectively. IgA level decreased not only at week 4 but also at week 8 compared to the baseline. IgA level in the GLBS group was 257 (99.25) and 263.5 (116) mg/dL at weeks 4 and 8, respectively, while IgA level in the placebo group was 232 (121.75) and 227.5 (172) mg/dL, respectively. Furthermore, the baseline IgE level in the GLBS and placebo groups was 25.7 (207.88) and 40.1 (144.83) mg/dL, respectively. IgE levels had increased in both groups at weeks 4 and 8: IgE level was 40 (174.2), 41.5 (97.35) mg/dL, and 50.7 (228.58), 50.7 (80.35) mg/dL in the GLBS and placebo groups at weeks 4 and 8, respectively. There were no significant differences between any of the Ig levels at baseline, week 4, or week 8 between groups (Figure 2). According to the subgroup analysis of cancer types, there were no significant differences in the Ig levels at any time points between groups in both breast and colorectal cancer (data not shown).

3.2.2 Complete blood counts

There were no significant differences in hemoglobin, hematocrits, monocytes, lymphocytes, neutrophils, eosinophils, basophils and platelets at baseline, weeks 4 and week 8 (Table 2). Even though there was no significant difference in neutrophils between the two groups, those in the GLBS group tended to sequentially increase. The absolute neutrophil count (ANC) and WBC levels in the GLBS group were significantly lower than those in the placebo group at baseline (p=0.017 and 0.017, respectively). Differences in WBCs in the GLBS group were significantly higher than the placebo group at week 8 (p<0.05), and the ANC in the GLBS group was more significantly different than the placebo group at both week 4 and week 8 (p=0.046 and 0.021, respectively) (Figures 3-4).

3.3 Safety profiles

3.3.1 Renal and liver functions

The baseline and follow-up values of renal and liver function are presented in Table 3. In this study, there was no significant effect on liver enzymes and renal function in either group at week 4 or week 8.

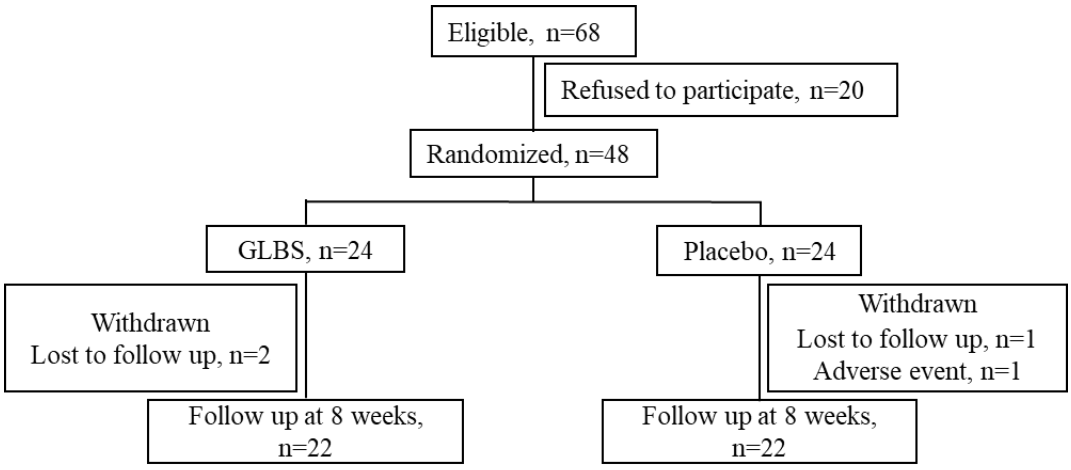

Figure 1. Progress of participants through the study

Table 1. Participant's characteristics

Characteristics	Frequency (percentage)		*p*-value
	GLBS (n=22)	Placebo (n=22)	
1. Gender*			0.488
Male	4(18.2)	7(31.8)	
Female	18(81.8)	15(68.2)	
2. Age (years)** (mean±SD)	55.32±2.57	53.82±2.41	0.549
3. Karnofsky performance status*			0.129
100	15(68.2)	9(41.0)	
90	7(31.8)	12(54.5)	
80	0(0)	1(4.5)	
4. Underlying disease***			0.546
No	12(54.5)	10(45.5)	
Yes	10(45.5)	12(54.5)	
5. Cancer***			0.750
Breast cancer	14(63.6)	15(68.2)	
Colorectal cancer	8(36.4)	7(31.8)	
6. Stage of cancer*			0.074
1	3(13.6)	4(18.1)	
2	15(68.2)	8(36.4)	
3	4(18.2)	10(45.5)	
7. Chemotherapy regimen prior the study*			0.982
5-FU/leucovorin	5(22.7)	3(13.6)	
AC	3(13.6)	3(13.6)	
AC-P	7(31.8)	7(31.8)	
FAC	3(13.6)	3(13.6)	
5-FU+oxaliplatin	3(13.6)	4(18.2)	
TC	1(4.5)	2(9.2)	
8. Concomitant treatments ***			0.621
No	11(50)	11(50)	
Radiation	1(4.5)	3(13.6)	
Radiation+hormonal drug	10(45.5)	8(36.4)	
9. Past 3 month's corticosteroids received prior to study (mg) (mean±SD)**	114.77±71.91	137.59±113.47	0.724
10. Compliance (mean±SD)**			
Week 4	97.32±4.38	96.05±4.89	0.131
Week 8	96.59±5.07	97.73±3.13	0.109

Note: *Comparison frequency between groups was determined using Fisher's exact test
**Comparison mean between groups was determined using independent t- test
***Comparison frequency between groups was determined using χ^2 test
AC = doxorubicin + cyclophosphamide; AC-P = doxorubicin + cyclophosphamide then paclitaxel; FAC = 5-fluorouracil + doxorubicin + cyclophosphamide; 5-FU = 5-fluorouracil; and TC = docetaxel + cyclophosphamide

3.3.2 Adverse events

The reported adverse events in the GLBS group were: dry mouth (n=1), oral wound (n=1), nausea (n=1), headache (n=1), and constipation (n=1), while pain (n=3), dry mouth (n=2), nausea (n=1), foot oedema (n=1), and constipation (n=1) were reported in the placebo group. All reported adverse events in both groups were mild, and there were no significant differences between the two groups (all $p>0.05$).

3.3.3 Quality of life

Changes in the total scores, physical well-being, social well-being, and functional well-being quality of life scores in the GLBS group were higher than those in the placebo group, but there were no significant differences in all domains of FACT-G (Figure 5).

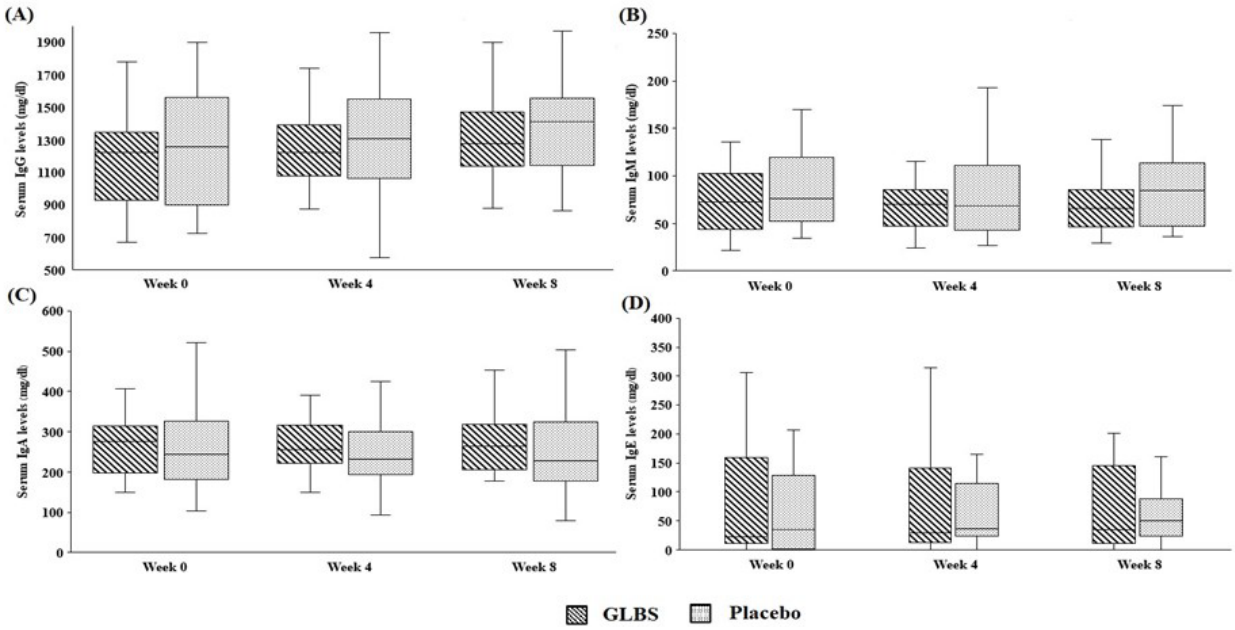

Figure 2. Serum immunoglobulin (Ig) levels at weeks 0 (baseline), 4, and 8 in the GLSB and placebo groups: (A) IgG, (B) IgM, (C) IgA, and (D) IgE

Table 2. Complete blood count in the GLBS and placebo groups at weeks 0, 4, and 8

	Normal range	**Week**	**Median (Interquartile range)**				***p*-value***
			GLBS (n=22)		**Placebo (n=22)**		
Hemoglobin	12.3-15.5 g/dL	0	11.8	(1.85)	11.0	(1.48)	0.621
		4	11.9	(2.35)	11.4	(1.58)	0.387
		8	12.2	(1.45)	12.0	(1.48)	0.387
Hematocrit	36.8-46.6%	0	35.9	(5.50)	34.7	(5.10)	0.860
		4	36.4	(5.60)	35.4	(4.50)	0.860
		8	36.9	(3.25)	36.4	(3.80)	0.860
White blood cells	4.24-10.18 $10^3/\mu L$	0	4.2	(2.36)	5.6	(2.88)	0.017
		4	4.4	(2.74)	5.1	(2.45)	0.245
		8	5.4	(3.03)	4.9	(1.58)	0.907
Monocyte	3.3-10.2%	0	9.0	(4.85)	8.5	(6.33)	0.985
		4	7.2	(2.10)	7.6	(2.00)	0.621
		8	6.9	(2.72)	6.3	(2.32)	0.860
Lymphocyte	21.1-42.7%	0	27.4	(11.40)	21.3	(9.20)	0.860
		4	26.5	(11.18)	25.8	(9.18)	0.860
		8	23.7	(21.83)	26.5	(14.05)	1.000
Eosinophil	0.4-7.2%	0	2.0	(2.50)	1.9	(2.93)	0.987
		4	3.2	(2.28)	3.1	(3.83)	0.860
		8	2.7	(1.82)	2.4	(3.68)	0.621
Neutrophil	48.1-71.2%	0	59.7	(13.70)	62.2	(14.28)	0.387
		4	62.3	(8.75)	62.1	(14.07)	0.987
		8	66.0	(16.97)	63.0	(9.85)	0.621
Basophil	0.1-1.2 %	0	0.5	(0.65)	0.5	(0.50)	0.987
		4	0.6	(0.32)	0.5	(0.35)	0.860
		8	0.6	(0.40)	0.4	(0.33)	0.387
ANC	>2500 mm^{-3}	0	2,687.5	(1,474.10)	3,792.4	(1,907.82)	0.017
		4	2,989.3	(1,365.31)	3,311.5	(1,599.26)	0.313
		8	3,227.7	(2,415.94)	3,057.4	(1,053.83)	0.796
Platelet	152-387 $10^3/\mu L$	0	266.0	(179.50)	273.5	(137.25)	0.860
		4	270.0	(116.0)	241.0	(112.25)	0.860
		8	247.0	(89.75)	230.5	(76.75)	0.621

Note: *Comparison median between groups was determined using the Kruskal Wallis H test.

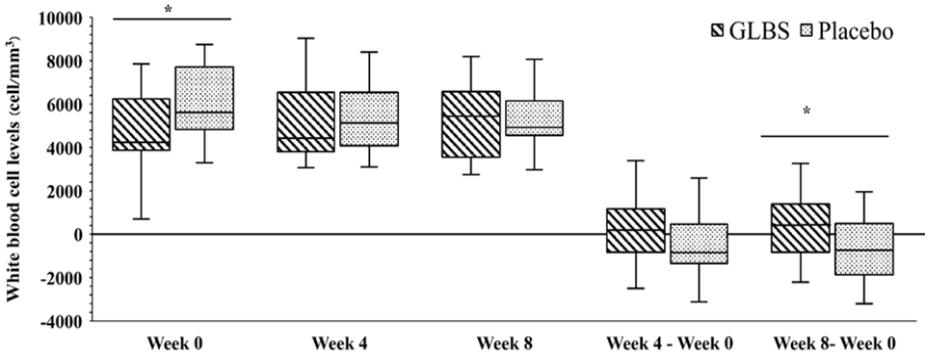

Figure 3. WBC levels at weeks 0, 4, and 8 and differentiation at week 4 and 8 compared with baseline WBC levels in the GLBS and placebo groups
Note: *Significant difference between groups was determined using the Kruskal Wallis H test (p<0.05).

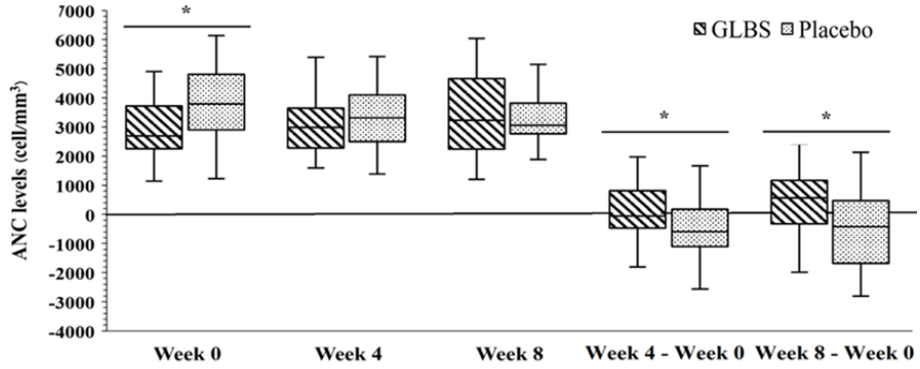

Figure 4. Absolute neutrophil count (ANC) levels at weeks 0, 4, and 8 and differentiation at weeks 4 and 8 compared with baseline ANC levels in the GLBS and placebo groups
Note: *Significant difference between groups was determined using the Kruskal Wallis H test (p<0.05).

4. DISCUSSION

GL, known as 'Lingzhi' or 'Reishi', is reported to be effective in inhibiting tumor growth as well as being an antioxidant and having an immunomodulatory profile. It produces a wide variety of bioactive substances, such as polysaccharides, triterpenoids, and long chain fatty acids (Boh et al., 2007). GLBS is a more active component than the fruiting body, mycelia, and unbroken spores (Yue et al., 2008). GL polysaccharide augmented both the percentage and size of B cells, in addition to inducing the production of Ig (Zhang et al., 2002; Boh et al., 2007). Another study also showed that GL extract significantly increased IgA in cow's blood (Liu et al., 2015) and in the Peyer's patches of mice (Kubota et al., 2018). Liu et al. (2020) reported that broilers taking GLBS 100, 200, and 500 mg/kg for 44 days had significantly increased serum IL-2, IgA, and IgG, while there was no significant change in serum IgM compared to the control. Moreover, a previous study showed glioma-bearing rats receiving GL polysaccharides for 2 weeks had significantly increased levels of IL-2, IL-10, IL-12, TNF-α, INF-γ, and cytotoxic activity of NK cells and T cells (Wang et al., 2018a). The function of GL polysaccharide may participate through TLR-4 and TLR-2, in addition to activated B lymphocyte-induced maturation protein (Blimp-1), leading to the production of Ig (Lin et al., 2006).

Research into GLBS properties and dosages in human Ig levels has been limited recently. This study showed that there was no significant difference in all types of Ig levels in breast or colorectal cancer patients after receiving GLBS 750 mg per day for 8 weeks. Chemotherapy had an impact not only on immune cells but also on hematologic cells. Prior research showed that the extracts from the GL fruiting body and GLBS, at 150 and 300 mg/kg for 40 days in Wister rats, increased hemoglobin and platelet levels, compared with baseline (Ahmed and Aslam, 2018). In addition, Zhao et al. (2018) showed that GL extract at dose of 11-46 mg per day for 28 days significantly increased WBCs in tumor-bearing mice receiving cisplatin. Furthermore, recombinant protein from the GL fruiting body (r-LZ8) significantly increased peripheral blood WBC levels, which correlated with the dosage (Lei et al., 2020). On the other hand, there was no significant difference in horses receiving GL mycelia at 30 g for 20 days (Lai et al., 2004) or Wister rats receiving GL fruiting bodies at 2-4 g per kg bodyweight for 28 days (Zhang et al., 2016). Studies of the hematology profile of GL have been unclear. This study showed that a supplement of 750 mg of GLBS given to cancer patients for 8 weeks following the completion of chemotherapy significantly increased total WBCs and ANC, while red blood cells, lymphocytes, neutrophils, basophils, eosinophils, monocytes, and platelets showed no significant difference, compared with baseline. IgE level tended to increase in the GLBS and placebo groups in this study. The likely explanation involves the short- and long-lived plasma cells that are responsible for IgE production. Short-lived plasma cells would be destroyed during chemotherapy, and only long-lived plasma cells would

persist (Whiteside et al., 2018). Moreover, IgE is related to hyper-sensitivity type I. The sequence of events in an allergic reaction consists of the production of IgE in response to an allergen, the binding of IgE to mast cells and basophils, and the release of mast cell mediators, such as histamine, and cytokines (Amarasekera, 2011). Although some IgE data were higher than the normal range, basophil, and eosinophil levels were not changed, indicating that these high IgE levels were not associated with a hypersensitivity reaction. In this study, the immunomodulating efficacy of GLBS was contrary to our hypothesis that all Igs and complete blood counts would increase after receiving GLBS, compared with the placebo. The reasons might be the different dosage from previous studies, such as Deng et al. (2021), who reported that 2 g of GLBS for 6 weeks stimulated T lymphocytes and cytokines in breast and lung cancer patients. The characteristics of patients in this study were also different from previous studies. Previous studies included patients who had ongoing chemotherapy treatment (Deng et al., 2021), while the patients in this study had already completed the chemotherapy course. Moreover, type of cancer and chemotherapy regimens might not affect the result, because this study was designed for pre- and post-analysis, which should remove con-founding factors from treatments.

Table 3. Renal and liver function tests in the GLBS and placebo groups at weeks 0, 4, and 8

	Normal range	Week	Median (Interquartile range)				*p*-value*
			GLBS (n=22)		Placebo (n=22)		
Liver function tests							
AST	5-34 unit/L	0	26.0	(21.75)	26.0	(15.00)	0.987
		4	24.0	(13.00)	22.0	(8.25)	0.621
		8	24.0	(21.00)	22.5	(9.50)	0.215
ALT	0-55 unit/L	0	21.5	(19.00)	20.0	(19.75)	0.987
		4	19.0	(21.50)	16.0	(12.50)	0.860
		8	24.0	(24.00)	18.5	(17.25)	0.621
ALP	40-150 unit/L	0	59.0	(34.00)	75.0	(28.75)	0.109
		4	65.5	(30.50)	73.5	(28.75)	0.648
		8	67.0	(27.25)	72.5	(17.75)	0.621
Albumin	3.5-5.2 g/dL	0	4.3	(0.63)	4.3	(0.35)	0.860
		4	4.3	(0.55)	4.3	(0.40)	0.860
		8	4.4	(0.43)	4.3	(0.43)	0.860
Total bilirubin	0.2-1.2 mg/dL	0	0.5	(0.55)	0.5	(0.40)	0.860
		4	0.6	(0.44)	0.5	(0.28)	0.621
		8	0.4	(0.31)	0.4	(0.25)	0.860
Renal function tests							
GFR (CKD-epi)	>60 mL/min/1.73m^2	0	98.0	(27.63)	94.2	(19.21)	0.860
		4	95.8	(20.88)	98.9	(15.84)	0.860
		8	96.5	(19.24)	93.5	(23.79)	1.000
BUN	6-20 mg/dL	0	9.4	(5.28)	8.5	(7.55)	0.621
		4	11.9	(3.50)	11.0	(6.30)	0.215
		8	12.0	(6.50)	9.9	(6.82)	0.387
Serum creatinine	0.45-0.75 mg/dL	0	0.7	(0.22)	0.7	(0.15)	0.860
		4	0.7	(0.25)	0.7	(0.11)	0.621
		8	0.7	(0.20)	0.7	(0.24)	0.987

Note: *Comparison median between groups was determined using the Kruskal Wallis H test; AST = aspartate transaminase; ALT = alanine transaminase; ALP = alkaline phosphatase; GFR = glomerular filtration rate; BUN = blood urea nitrogen

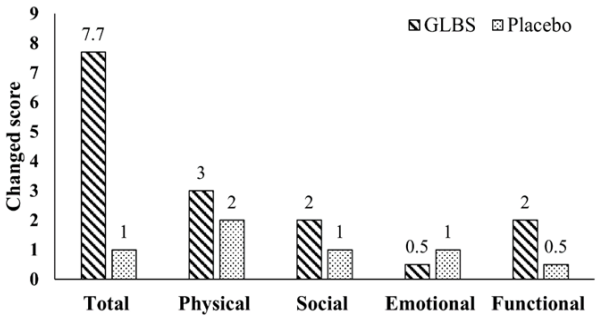

Figure 5. A comparison of changes in quality-of-life scores in the GLBS and placebo groups at week 8

In term of safety, GL has been shown to inhibit the activity of CYP2E1, CYP1A2, and CYP3A in hepatic metabolism (Wang et al., 2007). In this study, the researchers concerned about the interaction of GL and tamoxifen, because tamoxifen is metabolized via CYP2D6 and CYP3A4. A previous study showed no drug interaction between ether GL extract and tamoxifen at steady state (Atay et al., 2016). Renal and liver functions are important indicators for safety. Chiu et al. (2017) reported that AST and ALT levels were significantly decreased in healthy people receiving GL extract 225 mg/day for 6 months. Zhao et al. (2012) showed that liver and renal function were not significantly changed in breast cancer patients undergoing hormone therapy and GLBS of 3 g/day. Galor et al. (2007) found that GL was not only safe for the liver and kidneys but was also

protective for the liver. The hepatic and renal protective mechanisms of GL were mainly antioxidant (Jang et al., 2014; Zhong et al., 2015). On the other hand, there was a fatal fulminant hepatitis report in Thailand after taking 400 mg per day of GL extract (Wanmuang et al., 2007). Reported adverse events related to GLBS (Zhao et al., 2012; Wang et al., 2018b) were of mild severity, such as dry mouth, nausea, and vomiting, and no serious side effects were reported. GL has an antiplatelet aggregation profile dependent on the dosage. There was a report of bleeding during an operation with a patient using GL supplements (Shulman et al., 2014). Therefore, GLBS supplements should be avoided in patients with unstable liver function or any patients using antiplatelets or anticoagulants.

Important factors in the evaluation and selection of proper treatments include not only the treatment guidelines but also the patients' well-being. A variety of measures are available. One of the cancer-specific questionnaires is FACT-G, which is a core questionnaire that can be supplemented by a range of tumor, treatment, or symptom-specific modules as required. Preceding research using the European Organization for Research and Treatment of Cancer Quality of Life Questionnaire C30 (EORTIC QLQ-C30) showed that a supplement of GLBS 3 g/day for 4 weeks in breast cancer patients undergoing hormonal therapy significantly increased the quality of life with respect to the functional, emotional and cognitive domains, and the total quality of life score (Zhao et al., 2012). Jin et al. (2016) carried out a systematic review and showed that the use of GL in cancer patients had a positive impact on quality of life. This study showed no significant change in the quality of life in cancer patients who had completed chemotherapy and received GLBS for 8 weeks, in accordance with Gao et al. (2002), who reported that patients with advanced cancer receiving GL 5.4 g per day for 12 weeks had no significant change in the FACT-G score.

There was a limitation in this study, which could be addressed in future research. There were many *in vitro* GLBS efficacy studies but studies of GLBS in clinical trials were still limited. This study was conducted in breast or colorectal cancer patients who had completed chemotherapeutic treatment. Further studies should be performed in other conditions for example, with more types of cancer or with other concomitant cancer treatments.

5. CONCLUSION

A two-month supplementation of GLBS 750 mg per day in breast or colorectal cancer patients following the completion of chemotherapy significantly increased ANC and WBC levels; however, it was not shown to increase Ig significantly, compared with baseline. Although there were some mild adverse events, changes in renal and liver function tests were not found. In addition, the quality of life in the GLBS group tended to be better than that of the placebo group.

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REFERENCES

Ahmed, H., and Aslam, M. (2018). Effect of *Ganoderma lucidum* (Reishi) on hematological parameters in Wistar rats. *International Journal of Medical Research & Health Sciences*, 7(3), 151-157.

Amarasekera, M. (2011). Immunoglobulin E in health and disease. *Asia Pacific Allergy*, 1(1), 12-15.

Atay, S., Ak, H., Kalmis, E., Kayalar, H., and Aydin, H. H. (2016). Diverse effects of the Lingzhi or Reishi medicinal mushroom, *Ganoderma lucidum* (Agaricomycetes), in combination with tamoxifen citrate and doxorubicin in MCF-7 breast cancer cells. *International Journal of Medicinal Mushrooms*, 18(6), 489-499.

Benkeblia, N. (2015). *Ganoderma lucidum* polysaccharides and terpenoids: Profile and health benefits. *Journal of Food Nutrition Dietetics*, 1(1), 101.

Boh, B., Berovic, M., Zhang, J., and Bin, L. Z. (2007). *Ganoderma lucidum* and its pharmaceutically active compounds. *Biotechnology Annual Review*, 13, 265-301.

Cella, D. F., Tulsky, D. S., Gray, G., Sarafian, B., Linn, E., Bonomi, A., Silberman, M., Yellen, S. B., Winicour, P., Brannon, J. Eckberg, K., Lloyd, S., Purl, S., Blendowski, S., Goodman, M., Barnicle, M., Stewart, I., McHale, M., Bonomi, P., Kaplan, E., Taylor IV, S., Thomas Jr, C. R., and Harris, J. (1993). The functional assessment of cancer therapy scale development and validation of the general measure. *Journal of Clinical Oncology*, 11(3), 570-579.

Chiu, H. F., Fu, H. Y., Lu, Y. Y., Han, Y. C., Shen, Y. C., Venkatakrishnan, K., Golovinskaia, O., and Wang, C. K. (2017). Triterpenoids and polysaccharide peptides-enriched *Ganoderma lucidum*: A randomized, double-blind placebo-controlled crossover study of its antioxidation and hepatoprotective efficacy in healthy volunteers. *Pharmaceutical Biology*, 55(1), 1041-1046.

Deng, Y., Ma, J., Tang, D., and Zhang, Q. (2021). Dynamic biomarkers indicate the immunological benefits provided by Ganoderma spore powder in post-operative breast and lung cancer patients. *Clinical and Translational Oncology*, 23, 1481-1490.

Galor, S. W., Tomlinson, B., and Benzie, I. F. F. (2007). *Ganoderma lucidum* ('Lingzhi'), a Chinese medicinal mushroom: Biomarker responses in a controlled human supplementation study. *British Journal of Nutrition*, 91(2), 263-269.

Gao, Y., Zhou, S., Chen, G., Dai, X., and Ye, J. (2002). A phase I/II study of a *Ganoderma lucidum* (Curt.: Fr.) P. Karst. extract (Ganopofy) in patients with advanced cancer. *International Journal of Medicinal Mushrooms*, 4(3), 207-214.

Gao, Y., Zhou, S., Jiang, W., Huang, M., and Dai, X. (2003). Effects of Ganopoly® (a *Ganoderma lucidum* polysaccharide

extract) on the immune functions in advanced-stage cancer patients. *Immunological Investigations*, 32(3), 201-215.

Hack, C. C., Hackl, J., Hüttner, N. B. M., Langemann, H., Schwitulla, J., Dietzel-Drentwett, S., Fasching, P. A., Beckmann, M. W., and Theuser, A. K. (2018). Self-reported improvement in side effects and quality of life with integrative medicine in breast cancer patients. *Integrative. Cancer Therapies*, 17(3), 941-951.

Jang, S. H., Cho, S. W., Yoon, H. M., Jang, K. J., Song, C. H., and Kim, C. H. (2014). Hepatoprotective evaluation of *Ganoderma lucidum* pharmacopuncture: *In vivo* studies of ethanol-induced acute liver injury. *Journal of Pharmacopuncture*, 17(3), 16-24.

Jin, X., Beguerie, J. R., Sze, D. M. Y., and Chan, G. C. F. (2016). *Ganoderma lucidum* (Reishi mushroom) for cancer treatment. *Cochrane Database of Systematic Reviews*, 4, CD007731.

Katzung, B. G, Kruidering-Hall, M., and Trevor, A. J. (Eds.). (2019). Cancer chemotherapy. In *Katzung & Trevor's Pharmacology: Examination & Board Review*, 12th ed., pp. 450-462. New York: McGraw-Hill.

Kubota, A., Kobayashi, M., Sarashina, S., Takeno, R., Okamoto, K., Narumi, K., Furugen, A., Suzuki, Y., Takahashi, N., and Iseki, K. (2018). Reishi mushroom *Ganoderma lucidum* modulates IgA production and alpha-defensin expression in the rat small intestine. *Journal of Ethnopharmacology*, 214, 240-243.

Lai, S. W., Lin, J. H., Lai, S. S., and Wu, Y. L. (2004). Influence of *Ganoderma lucidum* on blood biochemistry and immunocompetence in horses. *The American Journal of Chinese Medicine*, 32(6), 931-940.

Lei, X., Zhi, C., Huang, W., Sun, X., Gao, W., Yin, X., Zhang, X., Liang, C., Zhang, H., and Sun, F. (2020). Recombinant *Ganoderma lucidum* immunomodulatory protein improves the treatment for chemotherapy-induced neutropenia. *Frontiers in Pharmacology*, 11, 956.

Lin, K. I., Kao, Y. Y., Kuo, H. K., Yang, W. B., Chou, A., Lin, H. H., Yu, A. L., and Wong, C. H. (2006). Reishi polysaccharides induce immunoglobulin production through the TLR4/TLR2-mediated induction of transcription factor Blimp-1. *Journal of Biological Chemistry*, 281(34), 24111-24123.

Liu, T., Zhou, J., Li, W., Rong, X., Gao, Y., Zhao, L., Fan, Y., Zhang, J., Ji, C., and Ma, Q. (2020). Effects of sporoderm-broken spores of *Ganoderma lucidum* on growth performance, antioxidant function and immune response of broilers. *Animal Nutrition*, 6(1), 39-46.

Liu, Y., Zhao, C., Lin, D., Lan, H., and Lin, Z. (2015). Effects of *Ganoderma lucidum* spent mushroom substrate extract on milk and serum immunoglobulin levels and serum antioxidant capacity of dairy cows. *Tropical Journal Pharmaceutical Research*, 14(6), 1049-1055.

Ratanatharathorn, V., Sirilerttrakul, S., Jirajarus, M., Silpakit, C., Maneechavakajorn, J., Sailamai, P., and Sirisinha, T. (2001). Quality of life, functional assessment of cancer therapy-general. *Journal of the Medical Association of Thailand*, 84(10), 1430-1442.

Sanodiya, B. S., Thakur, G. S., Baghel, R. K., Prasad, G., B., K. S., and Bisen, P. S. (2009). *Ganoderma lucidum*: A potent pharmacological macrofungus. *Current Pharmaceutical Biotechnology*, 10(8), 717-742.

Shulman, S., Sharma, S., Cracchiolo, B., Shubhangi, K., and Sifonios, A. (2014). Intraoperative bleeding associated with preoperative use of *Ganoderma lucidum* supplements. *Open Journal of Anesthesiology*, 4, 119-122.

Soonthornchareonnon. N. (2008). *Ganoderma lucidum* spore: The reason why its wall should be broken for medicinal use. *Journal of Thai Traditional Alternative Medicine*, 6(3), 313-321. [in Thai]

Verma, R., Foster, R. E., Horgan, K., Mounsey, K., Nixon, H., Smalle, N., Hughes, T. A., and Carter, C. R. (2016). Lymphocyte depletion and repopulation after chemotherapy for primary breast cancer. *Breast Cancer Research*, 18, 10.

Wang, C., Shi, S., Chen, Q., Lin, S., Wang, R., Wang, S., and Chen, C. (2018a). Antitumor and immunomodulatory activities of *Ganoderma lucidum* polysaccharides in glioma-bearing rats. *Integrative Cancer Therapies*, 17(3), 674-683.

Wang, H. G., Li, X., Cao, W. H., Li, J., and Wang, L. H. (2018b). A retrospective study of *Ganoderma Lucidum* spore powder for patients with epilepsy. *Medicine (Baltimore)*, 97(23), e10941.

Wang, L., Li, J., Zhang, J., Li, Z., Liu, H., and Wang, Y. (2020). Traditional uses, chemical components and pharmacological activities of the genus *Ganoderma* P. Karst.: A review. *Royal Society of Chemistry Advances*, 10, 42084-42097.

Wang, X., Zhao, X., Li, D., Lou, Y. Q., and Zhang, G. L. (2007). Effects of *Ganoderma lucidum* polysaccharide on CYP2E1, CYP1A2 and CYP3A activities in BCG-immune hepatic injury in rats. *Biological and Pharmaceutical Bulletin*, 30(9), 1702-1706.

Wanmuang, H., Leopairut, J., Kositchaiwat, C., Wananukul, W., and Bunyaratvej, S. (2007). Fatal fulminant hepatitis associated with *Ganoderma lucidum* (Lingzhi) mushroom powder. *Journal of Medical Association of Thailand*, 90(1), 179-181.

Whiteside, S., Markova, M., Chin, A., Lam, C., Dharmani-Khan, P., Modi, M., Khan, F., and Storek, J. (2018). Influence of chemotherapy on allergen-specific IgE. *International Archives of Allergy and Immunology*, 177, 145-152.

WHO. (2020). *All cancers*. World Health Organization. [Online URL: https://gco.iarc.fr/today/data/factsheets/cancers/39-All-cancers-fact-sheet.pdf] accessed on May 20, 2022.

Yue, G. G. L., Fung, K. P., Leung, P. C., and Lau, C. B. S. (2008). Comparative studies on the immunomodulatory and antitumor activities of the different parts of fruiting body of *Ganoderma lucidum* and *Ganoderma* spores. *Phytotherapy Research*, 22(10), 1282-1291.

Zhang, J., Gao, X., Pan, Y., Xu, N., and Jia, L. (2016). Toxicology and immunology of *Ganoderma lucidum* polysaccharides in Kunming mice and Wistar rats. *International Journal of Biological Macromolecules*, 85, 302-310.

Zhang, J., Tang, Q., Zimmerman-Kordmann, M., Reutter, W., and Fan, H. (2002). Activation of B lymphocytes by GLIS, a bioactive proteoglycan from *Ganoderma lucidum. Life Sciences*, 71(6), 623-638.

Zhao, H., Zhang, Q., Zhao, L., Huang, X., Wang, J., and Kang, X. (2012). Spore powder of *Ganoderma lucidum* improves cancer-related fatigue in breast cancer patients undergoing endocrine therapy: A pilot clinical trial. *Evidence-Based Complementary and Alternative Medicine,* 2012, 809614.

Zhao, R., Chen, Q., and He, Y. m. (2018). The effect of *Ganoderma lucidum* extract on immunological function and identify its anti-tumor immunostimulatory activity based on the biological network. *Scientific Reports*, 8, 12680.

Zhong, D., Wang, H., Liu, M., Li, X., Huang, M., Zhou, H., Lin, S., Lin, Z., and Yang, B. (2015). *Ganoderma lucidum* polysaccharide peptide prevents renal ischemia reperfusion injury via counteracting oxidative stress. *Scientific Reports*, 5, 16910.

Zhu, H. S., Yang, X. L., Wang, L. B., Zhao, D. X., and Chen, L. (2000). Effects of extracts from sporoderm-broken spores of *Ganoderma lucidum* on HeLa cells. *Cell Biology and Toxicology*, 16(3), 201-206.