Combination Treatment in Ulcerative Colitis Using 5-Aminosalysilic Acid (5-ASA) and Polysaccharide Peptide of Indonesian *Ganoderma lucidum* Mycelium Extract

Marcellus Simadibrata,**, Aditya Rachman*, Felix Budimutiar**, Paulus Simadibrata**, Murdani Abdullah*, Raja Mangatur Haloho*, Anthony Eka Wijaya*,***, Batara Bisuk*, Shabrina Maharani*, Dewi Mustikarani*, Daniel Martin Simadibrata***, Peter Sugita****

*Division of Gastroenterology, Department of Internal Medicine, Faculty of Medicine, Universitas Indonesia/Dr. Cipto Mangunkusumo General National Hospital, Jakarta
**Department of Internal Medicine, Abdi Waluyo Hospital, Jakarta
***Faculty of Medicine, Universitas Indonesia, Jakarta
****PT. Sahabat Lingkungan Hidup, Surabaya

Corresponding author:
Marcellus Simadibrata. Division of Gastroenterology, Department of Internal Medicine, Dr. Cipto Mangunkusumo General National Hospital. Jl. Diponegoro 71–73 Jakarta Indonesia. Phone: +62 816920448. E-mail: prof.marcellus.s@gmail.com

ABSTRACT

Background: *Inflammatory bowel disease (IBD) is idiopathic disease characterized by chronic inflammation of the gastrointestinal tract. Polysaccharide peptide of Ganoderma lucidum mycelium extract (PPGL) is considerably a good option for adjunctive therapy of IBD. This study aims to evaluate the benefit of PPGL in patients treated with 5-ASA.*

Method: *A retrospective observational cohort study was conducted to examine the medical records of 124 ulcerative colitis patients. There were 80 patients in intervention group who were treated with a combination of 5-ASA and PPGL, and there were 44 patients in the control group who were treated with 5-ASA only. Clinical and laboratory endpoints were observed at the baseline and after 30, 60, and 90 days. Clinical endpoints included abdominal pain, bloody diarrhea, aphthous stomatitis, and polyarthritis; meanwhile, laboratory endpoints included hemoglobin level, ESR, CRP, fecal calprotectin, MP2K, fecal culture, C. difficile culture, and colonoscopy results. Clinical trials conducted after patient enrollment with registry number ClinicalTrials.gov NCT04029649.*

Results: *On day-30, there was a significant difference between intervention group 45.6% and control group 2.3% in abdominal pain complaints ($p < 0.001$). Moreover, laboratory parameters of fecal calprotectin ($p < 0.001$), fecal MP2K ($p = 0.015$), and hemoglobin ($p < 0.001$) were considerably better in intervention group on day-30. These differences were consistently found on day-60 and 90.*

Conclusion: *The study implies potential correlation between PPGL administration and improvement of clinical and laboratory endpoints up to 90 days. A larger randomized, blinded, prospective study is required to confirm these effects in ulcerative colitis.*

Keywords: *ulcerative colitis, β-1,3/1,6-D-glucan, Ganoderma lucidum*

ABSTRAK

Latar belakang: *Inflammatory bowel disease (IBD) adalah penyakit idiopatik yang ditandai dengan inflamasi kronis dari traktus gastrointestinal. Polisakarida peptide dari Ganoderma lucidum mycelium ekstrak (PPGL) merupakan pilihan yang baik untuk terapi tambahan IBD. Penelitian ini bertujuan untuk mengevaluasi manfaat dari PPGL pada pasien yang diterapi dengan 5-ASA.*

Metode: *Penelitian retrospektif observasional kohort dilakukan dengan menganalisis rekam medis dari 124 pasien ulseratif kolitis. Terdapat 80 pasien pada kelompok intervensi dengan terapi kombinasi 5-ASA dan PPGL, dan terdapat 44 pasien pada kelompok kontrol dengan terapi hanya 5-ASA. Penilaian klinis dan laboratorium dilakukan setelah 30, 60, dan 90 hari. Penilaian klinis meliputi nyeri perut, diare berdarah, sariawan, dan poliartritis; sementara peniliain laboratorium meliputi level hemoglobin, ESR, CRP, fecal calprotectin, MP2K, kultur fekal, kultur C. difficile, dan hasil kolonoskopi. Uji klinis dilakukan dengan nomor registrasi ClinicalTrials.gov NCT04029649.*

Hasil: *Pada hari ke-30, terdapat perbedaan signifikan antara kelompok intervensi 45.6% dan kelompok control 2.3% pada keluhan nyeri perut ($p < 0.001$). Selain itu, parameter laboratorium fecal calprotectin ($p < 0.001$), fecal MP2K ($p = 0.015$), dan hemoglobin ($p < 0.001$) menujukkan hasil yang lebih baik pada kelompok intervensi pada hari ke-30. Perbedaan ini ditemukan secara konsisten pada hari ke-60 dan 90.*

Simpulan: *Penelitian ini menunjukan adanya korelasi potensial antara pemberian PPGL dengan perbaikan penilaian klinis dan laboratorium hingga hari ke-90. Penelitian prospektif, acak, dan tersamar yang lebih besar diperlukan untuk mengonfirmasi efek tersebut pada ulseratif kolitis.*

Kata kunci: *ulserative colitis, β-1,3/1,6-D-glucan, Ganoderma lucidum*

INTRODUCTION

Inflammatory bowel disease (IBD) is an idiopathic inflammatory disease characterized by chronic inflammation of the gastrointestinal tract and relapses.[1] IBD is classified into three types, namely ulcerative colitis (UC), Crohn's disease (CD), and intermediate colitis (IC). This classification is based on differences in the pathology and clinical manifestations that appear.[2] The most common clinical manifestations in IBD patients are chronic diarrhea with or without blood, abdominal pain, and weight loss. IBD is a disease with a high incidence and prevalence in Europe and North America. The average incidence of ulcerative colitis is 0.55 per 100,000 population.[3] The cause of UC is still unknown; however, many studies have found correlations to be a combination between genetic, environmental, diet, and autoimmune disorders.[4]

Treatment of UC consists of diet modification, lifestyle improvement, stress reduction, and medications. Available drugs that can be used to treat IBD symptoms include anti-inflammatory drugs 5-aminosalicylic acid (5-ASA), immunomodulators (azathioprine, mercaptopurine, methotrexate), and biological agents. Hanauer et al reported that monotherapy treatment with 5-ASA had about 30% remission. Therefore, adjunctive treatment of immunomodulators or biological agents is needed.[5]

An herbal alternative derived from *Ganoderma lucidum* mushroom can be used for adjunctive therapy in patients with ulcerative colitis. The polysaccharides inside the fungus have anti-inflammatory and immunomodulatory characteristics, which can reduce the production of TNF-α, IFN-γ, IL-17A. These inflammation factors are produced by colonic mucosal inflammation in patients with ulcerative colitis; therefore, *Ganoderma lucidum* might be helpful to be used as adjunctive therapy for ulcerative colitis.[6,7] In addition, a study done by Silva et al stated that *Ganoderma lucidum* could be used as an alternative dietary approach for the prevention of colitis-associated cancer.[8,9] This study aims to evaluate the added benefit of polysaccharide peptide of *Ganoderma lucidum* mycelium extract (PPGL) in patients treated with 5-ASA which is observed through the clinical and laboratory endpoints' improvements.

METHOD

Materials

The 5-ASA tablets given to the patients were enteric-coated 500 mg tablets made by the Falk foundation (Salofalk). PPGL is polysaccharide peptide of *Ganoderma lucidum* mycelium extract which is manufactured by PT. Sahabat Lingkungan Hidup –

Surabaya, Indonesia. 250 mg of PPGL contains 180 mg of β-1,3/1,6-D-glucans as active pharmaceutical ingredient (API). Chemical structure characterization analysis of β-1,3/1,6-D-glucan demonstrates that: (1) 1-D NMR spectroscopic profile is very similar to the beta-glucan of United States Pharmacopeia (USP) with reference standard for beta glucan: Cat. Number 1048288, Lot.Number FOK129; (2) Has a molecular weight > 3,755 kDa, with 54% purity; (3) Complex branching with a 2:1 bond ratio between β-1,3 main chain and β-1,6 branching. The structural characterization of β-1,3/1,6-D-glucan proves to be in accordance with the -1,3/1,6-D-glucan characterization that has a high potency as a strong immunomodulator, such as large molecular weight, complex branching structure, and triple helix solution conformation.[10]

Study Design

This single center retrospective observational cohort study was conducted at the Abdi Waluyo Hospital, Jakarta, Indonesia. We evaluated the correlation between 5-ASA and PPGL administration and improvement of clinical and laboratory results in patients with UC in comparison to the control group using 5-ASA only. All patients' data were collected in an anonymized fashion from the Abdi Waluyo hospital medical records with approval from the ethic committee of Abdi Waluyo Hospital. Clinical endpoints included aphthous stomatitis, polyarthritis, abdominal pain, and bloody diarrhea. Laboratory endpoints were hemoglobin levels, erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), fecal calprotectin, fecal M2PK, hyperemic colon on endoscopic examination, fecal culture test, *Clostridium difficile* toxin test (*C. diff* toxin). All of the data were assessed on baseline and after 30, 60, and 90 days post-treatment on either intervention or control groups.

Patients

Using total sampling technique, eligible patients were aged ≥ 18 with a history of inpatient or outpatient visits between January 2019 and December 2020 with a primary diagnosis of ulcerative colitis which was done with colonoscopy examination. Patients were treated with a combination of 5-ASA tablet 3 to 4 x 500 mg daily and 3 x 180 mg β-1,3/1,6-D-glucan daily or 5-ASA tablet 3 to 4 x 500 mg daily only.

Exclusion criteria were incomplete data on medical records, for example, no data on colonoscopy examination, clinical diagnosis of ulcerative colitis, diagnosis of Crohn's disease, incomplete treatment data on patients or inadequate follow up on patients, patient on other treatment besides 5-ASA and PPGL for ulcerative medications (biologic agents, immunomodulators, or steroids), and incomplete baseline data.

Data Collection

Data collection was done using predefined inclusion and exclusion criteria. Patients' data which were collected including age, sex, highest education, history of antibiotic usage, any comorbidities, ulcerative colitis symptoms and colonoscopy results before and after treatment, ulcerative colitis activity index score, laboratory results, and side effects of the treatment.

Laboratory Parameters Definition

We determined the laboratory parameters based on Abdi Waluyo Hospital's normal ranges. Low hemoglobin (Hb) is defined as less than 12 g/dL, high erythrocyte sedimentation rate (ESR) is defined as higher than 10 mm/hour, high C-reactive protein (CRP) is defined as higher than 11 mg/L, high fecal calprotectin is defined as higher than 50 µg/g from the stool sample. High fecal M2 pyruvate kinase (M2PK) is defined as M2PK results more than 4 U/mL.

Statistical analysis

Data analysis was done using IBM SPSS statistics for windows (version 25.0. Armonk, NY: IBM Corp) using the chi-square method with a significant value of $p < 0.05$, if the data distribution was not normal, Fisher exact test was used.

RESULTS

Selected Patients

A total of 275 patients with a primary diagnosis of ulcerative colitis were screened and 151 patients were not included in the study because they did not come back for post colonoscopy checkup, did not consume PPGL, laboratory results were not adequate to be included in the analysis, or only came once for a checkup (Figure 1).

In total, 124 patients were included in the study with 80 patients receiving 5-ASA and PPGL (intervention group) and 44 patients receiving 5-ASA treatment only (control group) (Table 1).

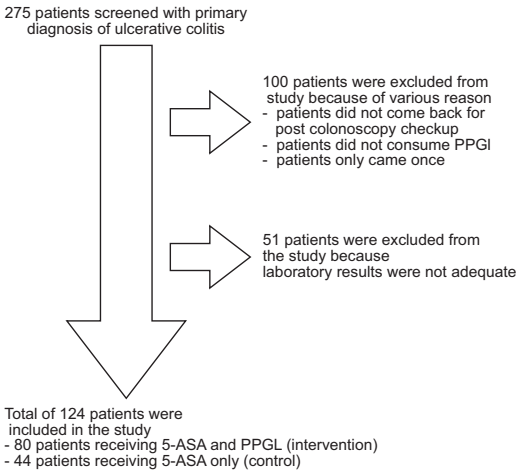

Figure 1. Prisma diagram

Table 1. Baseline characteristics of ulcerative colitis patients in Abdi Waluyo Hospital Jakarta Indonesia from 2019-2020

Variable	Intervention (5-ASA + PPGL) N = 80	Control (5-ASA) N = 44
Mean age, (SD) years	50.16 (1.6)	50 (2.0)
Male, n (%)	35 (43.8)	18 (40.9)
Education, n (%)		
High school	17 (21.3)	2 (4.5)
Vocational	1 (1.3)	0 (0)
Bachelor degree	61 (76.3)	49 (95.5)
Master degree	1 (1.3)	0 (0)
Occupation, n (%)		
Unemployed	22 (27.5)	11 (25.0)
Civil servant	3 (3.8)	6 (13.6)
Private employee	55 (68.8)	27 (61.4)
Presence of signs and symptoms, n (%)		
Bloody diarrhea	25 (31.3)	26 (59.1)
Abdominal pain	77 (96.3)	44 (100)
Aphtous stomatitis	5 (6.3)	8 (18.2)
Polyarthritis	9 (11.3)	18 (40.9)
Hemoglobin, n (%)		
Normal (≥ 12 g/dL)	67 (83.8)	32 (72.7)
Low (< 12 g/dL)	13 (16.3)	12 (27.3)
ESR, n (%)		
Normal (≤ 10 mm/hour)	47 (59.5)	38 (86.4)
High (> 10 mm/hour)	32 (40.5)	6 (13.6)
CRP, n (%)		
Normal (≤ 11 mg/L)	75 (94.9)	22 (50.0)
High (> 11 mg/L)	4 (5.1)	22 (50.0)
Fecal calprotectin, n (%)		
Normal (≤ 50 µg/g of stool)	14 (17.5)	3 (6.8)
High (> 50 µg/g of stool)	66 (82.5)	41 (93.2)
Fecal M2PK, n (%)		
Normal (≤ 4 U/mL)	27 (34.2)	14 (31.8)
High (> 4 U/mL)	52 (65.8)	30 (68.2)
Positive colonoscopy findings, n (%)		
Hyperemia	80 (100)	44 (100)
Erosions	75 (95)	37 (84.1)
Ulcer	7 (8.8)	6 (13.6)
Polyp	21 (26.3)	6 (13.6)
Fecal culture, n (%)		
Positive	0 (0)	(0)
Clostridium difficile toxin		
Reactive, n	1	0

5-ASA: 5-aminosalicylic acid; PPGL: polysaccharide peptide of *Ganoderma lucidum* mycelium extract; ESR: erythrocyte sedimentation rate; CRP: C-reactive protein; M2PK: M2 pyruvate kinase

Clinical End Points

Table 2. Clinical endpoints of patients with ulcerative colitis for 0, 30, 60, and 90 days post-treatment

Sign and symptoms	Day-0 n = 124			Day-30 n = 123			Day-60 n = 93			Day-90 n = 78		
	Intervention	Control	*p* value	Intervention	Control	*p* value	Intervention	Control	*p* value	Intervention	Control	*p* value
Bloody diarrhea, n (%)												
Not present	55 (68.8)	18 (40.9)		75 (94.9)	28 (63.6)		60 (100)	27 (81.8)		49 (100)	28 (96.6)	
Present	25 (31.3)	26 (59.1)	0.003	4 (5.1)	16 (36.4)	0.000	0 (0)	6 (18.2)	0.001*	0 (0)	1 (3.4)	0.372*
Abdominal pain, n (%)												
Not present	3 (3.8)	0 (0)		35 (45.6)	1 (2.3)		55 (91.7)	10 (30.3)		48 (98.0)	22 (75.9)	
Present	77 (96.3)	44 (100)	0.265*	43 (54.4)	43 (97.7)	0.000	5 (8.3)	23 (69.7)	0.000	1 (2.0)	7 (24.1)	0.003*
Aphtous stomatitis, n (%)												
Not present	75 (93.8)	36 (81.8)		78 (98.7)	41 (93.2)		60 (100)	33 (100)		49 (100)	29 (100)	
Present	5 (6.3)	8 (18.2)	0.41*	1 (1.3)	3 (6.8)	0.130*	0 (0)	0 (0)	–	0 (0)	0 (0)	–
Polyarthritis, n (%)												
Not present	71 (88.8)	26 (59.1)		75 (94.9)	28 (63.6)		58 (96.7)	21 (63.6)		49 (100)	20 (69.0)	
Present	9 (11.3)	18 (40.9)	0.000	4 (5.1)	16 (36.4)	0.000	2 (3.3)	12 (36.4)	0.000*	0 (0)	9 (31.0)	0.000*

**p*-value is calculated using Fisher exact test due to chi-square test assumption not fulfilled

Table 2 presents clinical endpoints for patients on the respective day of observation. Abdominal pain and aphthous stomatitis did not differ significantly in both intervention groups and control group. However, both polyarthritis and bloody diarrhea symptoms had a significant proportional difference in the intervention and control group.

On day-30, there was a significant difference between intervention group and control group in terms of abdominal pain complaints. 45.6% of patients who consumed PPGL as an adjuvant therapy reported that abdominal pain was not present since day 30 post-therapy compared to 2.3% of patients in control group ($x^2(1) = 25.19$; $p = 0.000$). However, this was not the case for aphthous stomatitis as there was no significant decrease in the patients' complaint ($p = 0.130$). Bloody diarrhea complaint in intervention group showed a significant difference compared to control group. 94.9% of patients who were given PPGL as adjuvant therapy did not complain of bloody diarrhea vs. 63.6% of patients who only received 5-ASA ($x^2(1) = 20.33$; $p = 0.000$). Polyarthritis complaint also showed a significant proportion difference in intervention group in comparison to control 94.9% vs. 63.6% of patients did not complain after 30 days of 5-ASA and PPGL ($x^2(1) = 20.33$; $p = 0.000$). However, these last two parameters might be confounding because the group proportion in day-0 showed a significant difference between intervention groups and control group before the start of therapy.

On day-60, intervention group still showed a significantly higher proportion of patients without abdominal pain complaint in comparison to control group. 91.7% of patients in intervention group were free of abdominal pain after 60 days of treatment with 5-ASA and PPGL ($x^2(1) = 38.10$; $p = 0.000$). Similar results were also shown in bloody diarrhea and polyarthritis complaints in intervention group that showed a higher percentage of complaint free compared to control group ($p = 0.001$) and ($p = 0.000$) respectively.

On day-90, intervention group still showed a significantly higher proportion of patients free from abdominal pain in comparison with patients in control group ($p = 0.003$, one-tailed); however, data calculation in this period was using Fisher exact test because chi-square assumption was not fulfilled. Bloody diarrhea and polyarthritis showed a significant difference in intervention and control group but due to the significant difference in sampling proportion on day-0, it was harder to determine the efficacy of PPGL in eliminating these symptoms.

Table 3 shows laboratory and colonoscopy results for enrolled patients on various post-treatment days either with a combination of 5-ASA and PPGL or 5-ASA only. On day-0, hemoglobin ($p = 0.143$), fecal calprotectin ($p = 0.098$), and fecal M2PK ($p = 0.790$) levels in both groups did not differ significantly. Therefore, these parameters were comparable and could be used to measure the association between PPGL administration and improvement of laboratory results. Other lab parameters such as ESR and CRP could not be used reliably to determine the effects of PPGL in ulcerative colitis patients because the starting samples were significantly difference in their proportion. Fecal culture, *Clostridium difficile*, and hyperemia from colonoscopy had 0 samples in either one of intervention or control group on day-0; therefore, the chi-square calculation could not be performed in these parameters. Erosion, ulceration, and polyp reports for colonoscopy also could not be used to determine the efficacy of PPGL in ulcerative colitis treatment due to inadequate follow-up.

On day-30, patients in the intervention group had a significantly higher proportion of normal hemoglobin values compared to control group ($x^2(1) = 12.19$; $p = 0.000$). Fecal calprotectin levels were also significantly lower in patients consuming PPGL as an adjuvant in comparison to control group. 50.6% of patients consuming 5-ASA and PPGL had normal calprotectin levels compared to 13.6% of patients who consumed 5-ASA only ($x^2(1) = 16.52$; $p = 0.000$). Fecal M2PK in the intervention group (65.8%) had a considerably larger proportion of patients with a normal level in comparison with control group (43.2%) ($x^2(1) = 5.94$; $p = 0.015$).

On day-60, only fecal calprotectin and MP2K levels exhibited a significantly higher proportion of patients with normal value in intervention group compared to control. 51.7% vs. 30.3% ($x^2(1) = 3.94$; $p = 0.047$) for fecal calprotectin and 68.3% vs. 42.4% ($x^2(1) = 5.91$; $p = 0.015$) for fecal MP2K. However, hemoglobin level in intervention and control group did not show a significant difference.

On day-90, fecal calprotectin levels in intervention group were still proportionally larger in patients with normal value in comparison to control group, 75.5% of patients on the intervention group vs. 51.7% of patients on the control ($x^2(1) = 4.638$; $p = 0.031$). Fecal M2PK also showed a significant difference between intervention and control group with 87.5% patients in intervention group demonstrating normal MP2K vs. 55.2% patients in control group ($x^2(1) = 10.165$; $p = 0.001$).

Laboratory and Colonoscopy Endpoints

Table 3.Laboratory and colonoscopy endpoints of patients with ulcerative colitis for 0, 30, 60, and 90 days post-treatment

Sign and symptoms	Day-0			Day-30			Day-60			Day-90		
	Intervention	Control	*p* value	Intervention	Control	*p* value	Intervention	Control	*p* value	Intervention	Control	*p* value
Hemoglobin, n (%)												
Normal (≥ 12 g/dL)	67 (83.8)	32 (72.7)		74 (93.7)	31 (70.5)		54 (90.0)	27 (81.8)		45 (91.8)	22 (75.9)	
Low (< 12 g/dL)	13 (16.3)	12 (27.3)	0.143	5 (6.3)	13 (29.5)	0.000	6 (10.0)	6 (18.2)	0.209*	4 (8.2)	7 (24.1)	0.054*
ESR, n (%)												
Normal (≤ 10 mm/hour)	47 (59.5)	38 (86.4)		48 (60.8)	33 (75.0)		48 (80.0)	23 (69.7)		46 (93.9)	23 (79.3)	
High (> 10 mm/hour)	38 (40.5)	6 (13.6)	0.002	31 (39.2)	11 (25.0)	0.110	12 (20.0)	10 (30.3)	0.263	3 (6.1)	6 (20.7)	0.059*
CRP, n (%)												
Normal (≤ 11 mg/L)	75 (94.9)	22 (50.0)		77 (98.7)	27 (61.4)		58 (98.3)	29 (87.9)		47 (97.9)	27 (93.1)	
High (> 11 mg/L)	4 (5.1)	22 (50.0)	0.000	1 (1.3)	17 (38.6	0.000	1 (1.7)	3 (12.1)	0.054*	1 (2.1)	2 (6.9)	0.316*
Fecal calprotectin, n (%)												
Normal (≤ 50 µg/g of stool)	14 (17.5)	3 (6.8)		40 (50.6)	6 (13.6)		31 (51.7)	10 (30.3)		37 (75.5)	15 (51.7)	
High (>50 µg/g of stool)	66 (82.5)	41 (93.2)	0.098	39 (49.4)	38 (86.4)	0.000	29 (48.3)	23 (69.7)	0.047	12 (24.5)	14 (48.3)	0.031
Fecal M2PK, n (%)												
Normal (≤ 4 U/mL)	27 (34.2)	14 (31.8)		52 (65.8)	19 (43.2)		41 (68.3)	14 (42.4)		42 (87.5)	16 (55.2)	
High (> 4 U/mL)	52 (65.8)	30 (68.2)	0.790	27 (34.2)	25 (56.8)	0.015	19 (31.7)	19 (57.6)	0.015	6 (12.5)	13 (44.8)	0.001
Fecal culture, n (%)												
Negative	80 (100)	44 (100)		78 (98.7)	44 (100)		60 (100)	33 (100)		49 (100)	29 (100)	
Positive	0 (0)	0 (0)	–	1 (1.3)	0 (0)	0.642*	0 (0)	0 (0)	–	0 (0)	0 (0)	–
C. diff toxin, n (%)												
Non reactive	2 (66.7)	0 (0)		1 (100)	0 (0)		1 (100)	0 (0)		1 (100)	0 (0)	
Reactive	1 (33.3)	0 (0)	–	0 (0)	0 (0)	–	0 (0)	0 (0)	–	0 (0)	0 (0)	–
Colonoscopy results, n (%)												
Hyperemia												
Not present	0 (0)	0 (0)		0 (0)	0 (0)		0 (0)	0 (0)		2 (100)	0 (0)	
Present	80 (100)	44 (100)	–	3 (100)	0 (0)	–	3 (100)	0 (0)	–	0 (0)	0 (0)	–
Erosion												
Not present	4 (5.0)	7 (15.9)		0	0		0 (0)	0 (0)		0 (0)	0 (0)	
Present	76 (95.0)	37 (84.1)	0.046*	3	0	–	3 (100)	0 (0)	–	2 (100)	0 (0)	–
Ulceration												
Not present	73 (91.3)	38 (86.4)		3 (100)	0 (0)		3 (100)	0 (0)		2 (100)	0 (0)	
Present	7 (8.8)	6 (13.6)	0.288*	0 (0)	0 (0)	–	0 (0)	0 (0)	–	0 (0)	0 (0)	–
Polyp												
Not present	59 (73.8)	38 (86.4)		3 (100)	0 (0)		2 (66.7)	0 (0)		2 (100)	0 (0)	
Present	21 (26.3)	6 (13.6)	0.103	0 (0)	0 (0)	–	1 (33.3)	0 (0)	–	0 (0)	0 (0)	–

ESR: erythrocyte sedimentation rate; CRP: C-reactive protein; M2PK: M2 pyruvate kinase

DISCUSSION

Ulcerative colitis patients usually come with chief complaint of chronic diarrhea with blood or abdominal pain. Our study showed significantly better results in patients treated with PPGL and 5-ASA in comparison to 5-ASA only, both in clinical and laboratory endpoints. Abdominal pain incidence was significantly less in the intervention group compared to the control group. Hemoglobin, fecal calprotectin, and MP2K parameters were also significantly improved in intervention group in comparison to control group.

Our findings with PPGL and 5-ASA could be compared with the effects of *Ganoderma lucidum* polysaccharide on colitis mice drug trial. The previous study was conducted on rat dextran sodium sulfate (DSS)–induced colitis. The study result that could be observed were a reduce disease activity index scores and an incline on cecal short chain fatty acid (SCFA) levels of DSS-Induced colitis rats. The previous study only provided drug trial information on mice; however, the results founded were similar with our current study, which is conducted in human. There were significant positive effects discovered both from the previous and current study on *Ganoderma lucidum* polysaccharide usage.[11]

Abdominal pain remained significantly lower in proportion in patients treated with a combination of 5-ASA and PPGL compared to 5-ASA only in any observed period. However, both bloody diarrhea and polyarthritis cannot be used properly to determine the efficacy of PPGL because of the significantly different proportion in the baseline sample.

Hemoglobin level was significantly become well in 30 days post-treatment in patients consuming PPGL in combination with 5-ASA compared to the control group. This might indicate less bloody diarrhea in these patients despite the fact that the study did not show correlation between PPGL consumption and the reduction of bloody diarrhea. Moreover, hemoglobin levels on day-60 did not significantly differ in both groups which might indicate that both treatments could control bloody diarrhea symptoms. However, combination therapy between 5-ASA and PPGL provided faster symptoms relief. This current finding showed that a combination between 5-ASA and PPGL was a better option in term of hemoglobin level compared to treatment with 5-ASA only or combination with sulphasalazine or azathioprine.[12]

Fecal calprotectin in the intervention group was significantly lower in the intervention group in any post-treatment observation period. Fecal calprotectin is a detectable protein in the stool that is directly proportional with neutrophils number in the intestines, consequently a lower calprotectin level correlates with less inflammation inside the colon.[1] Furthermore, the current finding of lower number of fecal calprotectin in the intervention group was aligned with aprevious study on fecal calprotectin level reduction on patient using PPGL.[13] Based on the current finding and some previous studies, it could be concluded that PPGL has immunomodulatory and anti-inflammatory properties which can reduce inflammation inside the colon which was proven by the fall off fecal calprotectin level in the intervention group.[1,13]

MP2K is another parameter that was found to be significantly affected by PPGL administration. In either 30, 60, or 90 days post-treatment it can be seen that the number of patients who have normal M2PK levels is considerably higher compared to control group. PPGL were reducing the MP2K value in intervention group. These findings were aligned with the result of the previous research on fecal M2-pyruvate kinase, which observed a higher elevation in MP2K levels in active, compared to inactive, disease for CD and UC.[14] Moreover, there was another study which proved that MP2K is a useful indicator to measure the level of disease activity in patients with IBD.[15] In conclusion, it was clear that a reduction of MP2K values is connected to the reduction of ulcerative colitis activity.

ESR is affected by multiple factors including smoking, age, sex, metabolic disease such as diabetes mellitus.[16] C-reactive protein is also affected by infection, thalassemia, and other diseases which trigger inflammation because it is an acute phase reactant.[17] Therefore, ESR and C-reactive protein werenot considered as suitable parameters to measure the treatment efficacy of PPGL in ulcerative colitis treatment due to significant differences in population baseline between intervention and control group.[18]

The *Clostridium difficile* finding as the side effect of 5-ASA and PPGL was also not achievable due to limited data on medical records on days 0, 30, 60, and 90 of treatment. In colonoscopy examination, hyperemia was found in all ulcerative colitis patients, followed by erosions (91.1%), polyps (21.8%), and ulcers (10.5%). These findings are due to the inflammatory process triggered by IBD.[19] However, colonoscopy follows up results was not satisfactory because in most patients the procedure is only done once, presumably in order to diagnose ulcerative colitis the colonoscopy procedure is expensive, so the patients usually do follow up by lab or evidence of clinical

result improvement.[20] Therefore, the efficacy of 5-ASA and PPGL on colonoscopy results cannot be analyzed due to limited or incomplete medical record data.

Since impaired innate and adaptive immune responses that lead to intestinal chronic inflammation are involved in ulcerative colitis pathogenesis, there may be an opportunity for PPGL to be used as adjuvant therapy due to its role as an immunomodulative properties. β-1,3/1,6-D-glucan as API of PPGL is able to bind to 5 receptors that belong to the immune cells: Dectin-1, Toll-like receptor (TLR), complement receptor-3 (CR-3), lactocylceramide, and scavenger receptor.[13] PPGL has demonstrated in pre-clinical and several randomized controlled trial as an anti-inflammatory and anti-oxidation agent through its ability to reduce the levels of IL-6, TNF-α, CRP, MDA, and H_2O_2 significantly ($p < 0.05$) after the administration of this β-1,3/1,6-D-glucan as adjuvant therapy in cardiovascular diseases with a dose of 540 mg daily for 90 days, by modulating the immune cells.[21,22] Therefore, it could be expected PPGL is able to prevent and repair the damage of intestinal barrier in ulcerative colitis.

This study, however, was not able to prove PPGL's efficacy in treating patients with ulcerative colitis because of the nature of retrospective study. A prospective study with larger sample with randomization and blinding is required to ensure the efficacy of PPGL, in addition, significant difference in several clinical and laboratory act as a confounding factor.

This study provides evidence of added clinical benefits of PPGL for patients with ulcerative colitis who were previously treated with 5-ASA. Abdominal pain complaints, hemoglobin level, fecal calprotectin, and fecal MP2K were significantly better in patients treated with PPGL and 5-ASA. However, a larger randomized, blinded, prospective study is required to prove these effects in a larger group of ulcerative colitis patients.

CONCLUSION

The study showed a correlation between PPGL administration and improvement of clinical and laboratory endpoints, mainly reduction of abdominal pain complaints, normalization of HB levels, fecal M2PK, and fecal calprotectin. Administration of PPGL as adjunctive therapy may be clinically beneficial in patients with ulcerative colitis. Larger studies are required to replicate the improvement in clinical and surrogate laboratory parameters.

REFERENCES

1. Bjarnason I. The use of fecal calprotectin in inflammatory bowel disease. Gastroenterol Hepatol 2017;13:53–6.
2. Ye Y, Pang Z, Chen W, Ju S, Zhou C. The epidemiology and risk factors of inflammatory bowel disease. Int J Clin Exp Med 2015;8:22529–42.
3. Ng SC, Tang W, Ching JY, Wong M, Chow CM, Hui AJ, et al. Incidence and phenotype of inflammatory bowel disease based on results from the Asia-Pacific Crohn's and colitis epidemiology study. Gastroenterology 2013;145:158–65.
4. Makmun D. In Fauzi A, Maulahela H, Pribadi RR, eds. Konsensus Nasional Penatalaksanaan Inflammatory Bowel Disease [IBD] di Indonesia (Revisi 2019). Jakarta: PIPInterna. 2019.p.1–42.
5. Hanauer S, Schwartz J, Robinson M, Roufail W, Arora S, Cello J, et al. Mesalamine capsules for treatment of active ulcerative colitis: results of acontrolled trial. Pentasa Study Group. Am J Gastroenterol 1993;88:1188–97.
6. Liu C, Dunkin D, Lai J, Song Y, Ceballos C, Benkov K, et al. Anti-inflammatory effects of *Ganoderma lucidum* triterpenoid in human Crohn's disease associated with downregulation of NF-κB signaling. Inflamm Bowel Dis 2015;21:1918–25.
7. Wang Y, Ouyang Q. Ulcerative colitis in China: retrospective analysis of 3100 hospitalizedpatients. J Gastroenterol Hepatol 2007;22:1450–5.
8. Sliva D, Loganathan J, Jiang J, Jedinak A, Lamb JG, Terry C, et al. Mushroom *Ganoderma lucidum* prevents colitis-associated carcinogenesis in mice. PLoS One 2012;7:e47873.
9. Erbayrak M, Turkay C, Eraslan E, Cetinkaya H, Kasapoglu B, Bektas M. The role of fecal calprotectin in investigating inflammatory bowel diseases. Clinics 2009;64:421–5.
10. Sugita P, Sargowo D. Abstract 366: beta-1,3 / 1,6-D-glucan chemical structure characterization of Indonesian *Ganoderma lucidum* mycelium extract. Arterioscler Thromb Vasc Biol 2019;39:A366.
11. Xie J, Liu Y, Chen B, Zhang G, Ou S, Luo J, et al. *Ganoderma lucidum* polysaccharide improves rat DSS-induced colitis by alteringcecal microbiota and gene expression of colonic epithelial cells. Food Nutr Res 2019;63:x.
12. Gasche C, Lomer MCE, Cavill I, Weiss G. Iron, anaemia, and inflammatory bowel diseases. Gut 2004;53:1190–7.
13. Lin ZB. Cellular and molecular mechanisms of immuno-modulation by *Ganoderma lucidum*. J Pharmacol Sci 2005;99:144–53.
14. Chung-Faye G, Hayee B, Maestranzi S, Donaldson N, Forgacs I, Sherwood R. Fecal M2-pyruvate kinase (M2-PK): a novel marker of intestinal inflammation. Inflamm Bowel Dis 2007;13:1374–8.
15. Roszak D, Gałęcka M, Cichy W, Szachta P. Determination of faecal inflammatory marker concentration as a noninvasive method of evaluation of pathological activity in children with inflammatory bowel diseases. Adv Med Sci 2015;60:246–52.
16. Tishkowski K, Gupta V. Erythrocyte sedimentation rate. In: StatPearls [serial online]. Treasure Island (FL): StatPearls Publishing Treasure Island: StatPearls 2022. Available from: https://www.ncbi.nlm.nih.gov/books/NBK557485/.
17. Nehring SM, Goyal A, Patel BC. C reactive protein. In: StatPearls [serial online]. Treasure Island (FL): StatPearls Publishing Treasure Island: StatPearls 2022. Available from: https://www.ncbi.nlm.nih.gov/books/NBK441843/.

18. Chen P, Zhou G, Lin J, Li L, Zeng Z, Chen M, et al. Serum biomarkers for inflammatory bowel disease. Front Med 2020;7:1–17.
19. Lee JM, Lee KM. Endoscopic diagnosis and differentiation of inflammatory bowel disease. Clin Endosc 2016;49:370–5.
20. Henry SG, Ness RM, Stiles RA, Shintani AK, Dittus RS. A cost analysis of colonoscopy using microcosting and time-and-motion techniques. J Gen Intern Med 2007;22:1415–21.
21. Chan GCF, Chan WK, Sze DMY. The effects of beta-glucan on human immune and cancer cells. J Hematol Oncol 2009;2:25.
22. Heriansyah T, Nurwidyaningtyas W, Sargowo D, Tjahjono CT, Wihastuti TA. Polysaccharide peptide (PSP) *Ganoderma lucidum*: a potential inducer for vascular repair in type 2 diabetes mellitus model. Vasc Health Risk Manag 2019;15:419–27.