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Efficacy and Safety of Brazilian Green Propolis in Biochemically Recurrent Prostate Cancer after Radical Prostatectomy: A Single-Arm Phase II Study

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Abstract: Background: Radiation or hormonal therapy is considered for prostate cancer patients with biochemical recurrence (BCR) after radical prostatectomy (RP). However, these therapies have their own complications. To delay the start of these therapies, we investigated the efficacy and safety of Brazilian green propolis for the treatment for BCR after RP. Materials and Methods: This single-center, single-arm open trial included 22 patients who experienced BCR after RP between 2016 and 2019. The patients received nine softgels of Brazilian green propolis (containing 40 mg propolis per capsule) daily for 6 months. The primary outcome was the prostate-specific antigen (PSA) response rate. The secondary outcomes included progression-free time, PSA slope (1/PSA doubling time) response rate, quality of life, and safety profile. Results: The PSA response rate was 0%. The mean PSA slopes before and after baseline were 0.12 month^{-1} and 0.08 month^{-1}, respectively. Fifteen patients (68%) showed a decreased PSA slope after treatment. There were no negative effects on quality of life or serious adverse events leading to treatment discontinuation. Conclusion: There was no significant anticancer response in patients who received Brazilian green propolis. However, the PSA slope was decreased after propolis administration. Further, Brazilian green propolis may be safely consumed by patients.

Keywords: biochemical recurrence; Brazilian green propolis; prostate cancer

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1. Introduction

Approximately 35% of men experience biochemical recurrence (BCR) of prostate cancer (PCa) after radical prostatectomy (RP), despite early detection of the primary tumor using a serum prostate-specific antigen (PSA) test and improvements in surgical techniques [1]. Most post-RP recurrences are discovered by an initial increase of PSA without radiological and clinical recurrence [2]. The recently introduced standard treatment against BCR after RP is salvage radiation (SR) therapy for cases in which it is uncertain whether the site of recurrence is local, distant, or both [3]. However, if the recurrent lesion is metastatic, there would be little or no benefit of SR. Patients with metastatic disease may benefit from systemic therapies, the most common being salvage hormonal therapy.

Recently, three large multicenter trials (RAVES, GETUG-AFU17, and RADICALS RT) have examined the optimal timing for postoperative radiation in high-risk PCa patients [4–6]. Patients with postoperative PSA $\leq$ 0.10 ng/mL could be placed under observation without early SR until their PSA levels reached 0.20 ng/mL. Pfister et al. reported that patients treated with early SR with non-higher PSA concentrations (PSA $\leq$ 0.50 ng/mL) have a significantly improved BCR-free survival rate compared with those receiving delayed SR with higher PSA concentrations (PSA > 0.50 ng/mL) [7]. However, the three trials did not provide information regarding the effectiveness of delayed SR. The benefit of early hormonal therapy for nonmetastatic PCa relapses remains unknown [8]. Considering the risk of its associated side effects and lack of sufficient clinical evidence, early hormonal therapy should be reserved for patients with a high risk of disease progression, such as those with a short PSA doubling time (PSA-DT) at relapse or a high Gleason score [8]. Therefore, there persists a need for new treatment strategies that are effective and non-toxic for patients with BCR post-RP and PSA levels between 0.20 ng/mL and 0.50 ng/mL.

Complementary and alternative medicine (CAM) refers to a broad collection of self-care and practitioner-based practices that have been used outside of conventional healthcare. People use CAM worldwide; indeed, 10–76% of the general population reportedly used CAM within the past 12 months [9]. Among adults with cancer, 35.1% of them reported using some form of CAM within the past year in the United States [10]. Propolis is a sticky and dark-colored material produced by honeybees that collect various constituents, such as tree exudates, leaf buds, and other parts of plants [11]. Since ancient Greek and Egyptian periods, propolis has been used in various fields, particularly in traditional medicine as a disinfectant and antiseptic for cutaneous infections [12,13]. The molecular composition of propolis is primarily dependent upon its geographical and floral origins [11]. Brazilian green propolis has been reported to have anticancer activity, and its active components are experimentally revealed to be cinnamic acid derivatives, such as artepillin C, baccharin, and drupanin [14,15]. Endo et al. has recently reported that among existing aldo-keto reductase (AKR) 1C3 inhibitors, baccharin was the most selective inhibitor [16]. AKR1C3 belongs to the AKR superfamily of proteins and is involved in intratumoral androgen biosynthesis in prostate and breast cancer [17]. Additionally, AKR1C3 decreases levels of antiproliferative prostaglandin (PG) D_2 and 15-deoxy-Δ-PGJ_2 [17]. Furthermore, AKR1C3 is overexpressed in prostate tissue in some PCa patients [18]. These considerations prompted us to use propolis as a treatment against BCR after RP for localized PCa. The purpose of this study was to investigate the efficacy and safety of Brazilian green propolis in patients with BCR of localized PCa [15,19].

2. Material and Methods

2.1. Ethics Statement

The protocol of this study was reviewed and approved by the institutional review boards and ethics committees of Kyoto University Graduate School of Medicine (institutional review board number: YC1196-2) and registered in the UMIN Clinical Trials Registry (registration number: UMIN 000023451). We conducted all experiments involving human subjects according to the principles expressed in the Declaration of Helsinki and received written informed consent from all the patients. Enrollment and data management were performed in an independent data center at the Translational Research Center, Kyoto University Hospital.

2.2. Patient Population

At our Institution, we performed 1051 radical prostatectomies between October 1999 to October 2019. The study was conducted between November 2016 and October 2019, and we recruited men among 132 patients who had been a BCR after radical prostatectomy during the study periods. BCR was defined as a serum PSA value exceeding 0.2 ng/mL, obtained from a sequence of elevated PSA values, and derived from samples collected at a minimum of 2-week intervals after RP. Other eligibility criteria for enrollment in this

study were as follows: (i) age below 85 years; (ii) no clinical recurrence detected by imaging analysis, such as computed tomography, magnetic resonance imaging, bone scintigraphy, and Fluorodeoxyglucose-position emission tomography; (iii) ≥4 weeks from RP or SR; (iv) non-recipient of salvage hormonal therapy after RP; (v) absence of severe complications and abnormal laboratory findings, such as white blood cell count <3000/mm^3, hemoglobin <90 g/L, platelet count <75,000/mm^3, serum glutamic oxaloacetic transaminase >90 IU/L, serum glutamic pyruvic transaminase >100 IU/L, and serum creatinine >2.0 mg/dL; (vi) performance status (defined in Common Toxicity Criteria, Version 2.0) [20] between 0 and 2; (vii) documented informed consent provided after an explanation of the research purposes; (viii) absence of allergy to bee products and other allergic conditions, such as atopic dermatitis, chronic recurrent eczema, or asthma.

2.3. Summary of the Study and the Collected Data

This study was a single-center, single-arm open trial using Brazilian green propolis as the administered intervention. In the absence of adverse effects, the patients received nine softgel capsules of Brazilian green propolis products (API Co., Ltd. Gifu, Japan), containing 40 mg propolis per capsule with ethanol extraction, per day for six months and submitted the records of daily intake to physicians. Intake compliance of less than 80% resulted in exclusion from the study. Serum PSA value, complete blood cell count, and serum chemistry were examined at study baseline and 1, 2, 3, 4, and 6 months of follow-up. Complete blood cell count and serum chemistry, excluding serum PSA, were also examined two weeks after study initiation to check for acute toxicity. Serum sex hormone values (luteinizing hormone, follicle-stimulating hormone, total testosterone, and estradiol) were examined at baseline and 1, 3, and 6 months of follow-up. To evaluate the Patient-Reported Outcomes (PRO) assessment, we collected the FACT-P (Functional Assessment of Cancer Therapy-Prostate) score [21]. This questionnaire comprised five domains: physical, social, emotional, functional, and prostate-specific concerns; the maximum scores per domain were 28, 32, 24, 28, and 48 points, respectively. The "general" domain was the sum of all domains excluding prostate-specific concerns and had a maximum score of 112 points. Any adverse effects (allergic reaction, fatigue, or other symptoms) were recorded according to the National Cancer Institute Common Toxicity Criteria for Adverse Events, Version 3.0 [22]. After the initial six-month protocol, the patients who decided to continue the study for additional six months received Brazilian green propolis. During this extended period, we continued the monitoring of intake compliance, serum PSA level, and adverse effects.

2.4. Primary and Secondary Endpoints

The primary endpoint of this study was the PSA response rate, which was defined according to the prostate cancer working group 1 guideline [23]. The PSA response rate was defined as the proportion of patients who achieved >50% reduction in PSA value compared with the baseline PSA value. We also evaluated the following secondary endpoints: (i) duration of PSA response: the period from when a ≥50% reduction in PSA value was noted to when PSA progression was confirmed; (ii) PSA progression-free time: the duration within which a 25% increase in PSA value from baseline was observed; (iii) PSA slope response rate: the proportion of patients whose PSA slope decreased during the six months of the study compared with that measured six months before the study. The PSA slope, a reciprocal of PSA-DT [24], was defined as an approximate PSA increase over the study period. We used the PSA-DT calculator provided by Memorial Sloan–Kettering Cancer Center (https://www.mskcc.org/nomograms/prostate/psa_doubling_time, accessed on 1 January 2021). The PSA slope ratio was defined as the PSA slope after entry divided by the one before entry. A PSA slope ratio <1 indicated a slower PSA velocity after entry compared with before entry. Therefore, the PSA slope response rate was the proportion of patients with a PSA slope rate of <1. (iv) Testosterone response rate: the proportion of patients with a ≥20% reduction in serum testosterone value from that measured at baseline; (v) correlation between PSA slope response rate and immunohistochemistry score

in AKR1C3, ERG, and AR; (vi) the effect on PRO measured by FACT-P questionnaire; and (vii) the safety profile of Brazilian green propolis.

2.5. Immunohistochemistry

All patients in this study underwent RP. Immunohistochemistry was performed using the RP specimens of patients who had not undergone neoadjuvant hormonal therapy. We used an anti-AKR1C3 antibody (at a dilution of 1:200; ab49680, Abcam, Cambridge, UK), an anti-AR antibody (at a dilution of 1:400; CST#5153, Cell Signaling Technology, Danvers, MA, USA), and an anti-ERG antibody (at a dilution of 1:1000; ab92513, Abcam). Immunohistochemistry for AKR1C3 and AR was performed using an automated immunohistostaining device: the Ventana Discovery Ultra system (Roche Diagnostics, Basel, Switzerland). Immunohistochemistry for ERG was performed manually using Avidin-Biotin Complex (Vector Laboratories, Newark, CA, USA). We determined a staining score—the sum of a proportion score and an intensity score—for each tumor. The AKR1C3 staining score was evaluated in the most immunostained cells. The AKR1C3 proportion score was graded as follows: <1% (score 0), 1–10% (score 1), 11–33% (score 2), 34–66% (score 3), and >67% (score 4). The AKR1C3 intensity score was graded as follows: none (score 0), weak (score 1), intermediate (score 2), and strong (score 3) [18]. The AR and ERG staining scores were evaluated in the nuclei of carcinoma cells. AR and ERG proportion scores were graded as follows: none (score 0), 0–1% (score 1), 1–10% (score 2), 11–33% (score 3), 34–66% (score 4), and >67% (score 5). The AR and ERG intensity scores were graded according to average staining intensity as none (score 0), weak (score 1), intermediate (score 2), and strong (score 3) [25]. A pathologist and two urologists evaluated all the staining scores of all specimens.

2.6. Statistical Design and Analyses

This was a single-arm, Fleming's four-stage phase II study [26] with a one-sided significance level of 10% and 90% power for a null PSA response rate of 5% versus an alternative of 20%. If 3 or more confirmed PSA responses were observed among 10 patients in the first stage, the trial would be terminated and considered positive. Otherwise, an additional 10 patients would be enrolled in the second stage. In the second stage with 20 patients, the trial would be terminated and considered positive if 4 or more confirmed PSA responses were observed, and terminated for futility if no confirmed PSA responses were observed. Otherwise, an additional 10 patients would be enrolled in the third stage. Similarly, in the third stage with 30 patients, the trial would be terminated and considered positive if 4 or more confirmed PSA responses were observed, or terminated for futility if 2 or less confirmed PSA responses were observed. Finally, the trial would be considered positive if 5 or more confirmed PSA responses were observed among a total of 40 patients in the fourth stage.

The 95% confidence intervals (Cl) for PSA slope response rate and testosterone response rate were calculated using the Clopper–Pearson method. The clinical characteristics of patients with a PSA slope ratio <1 and those with a PSA slope ratio $\geq$1 are summarized using frequencies and percentages for categorical variables and means and standard deviations (SDs) for continuous variables. These were then compared using the Student's *t*-test for continuous variables and Fisher's exact test or the Cochran–Armitage test for categorical variables. The changes in the FACT-P score were investigated using a linear mixed-effects model. We included baseline and time predictors in the models as fixed effects. We used a random intercept to take into account the heterogeneity across subjects and the correlation induced by having repeated observations on the same subjects. All statistical analyses were performed using SAS 9.4 software (SAS Institute, Cary, NC, USA).

3. Results

3.1. Baseline Characteristics of the Patients

Based on the second interim evaluation, the trial was terminated because none of the patients responded; however, two additional patients had consented to treatment during this discussion period. Therefore, a total of 22 patients were enrolled in this trial.

Three patients had received neoadjuvant hormonal therapy before RP, and one underwent SR five years before study entry. Table 1 shows patient characteristics in detail. The mean PSA values at initial diagnosis and baseline were 9.4 (SD: 4.9) ng/mL and 0.34 (SD: 0.21) ng/mL, respectively. The mean period until BCR and study entry was 4.2 (SD: 2.4) years and 5.2 (SD: 2.7) years, respectively. Five patients had a history of some CAM usage except propolis before entry. No variant histology at the time of RP pathological examination was identified in all patients.

Table 1. Patients' characteristics.

Factor	Group	N = 22
Age (year old)	N	22
	Mean (SD)	71.0 (5.8)
BMI (kg/m^2)	N	22
	Mean (SD)	22.7 (2.6)
Initial PSA (ng/mL)	N	22
	Mean (SD)	9.4 (4.9)
PSA at study entry (ng/mL)	N	22
	Mean (SD)	0.34 (0.21)
Biopsy Gleason score	N	22
	3 + 3	5 (22.7%)
	3 + 4	6 (27.3%)
	4 + 3	3 (13.6%)
	4 + 4	6 (27.3%)
	3 + 5	0 (0.0%)
	4 + 5	1 (4.5%)
	5 + 4	1 (4.5%)
Clinical T stage	N	22
	T1c	7 (31.8%)
	T2a	10 (45.5%)
	T2b	2 (9.1%)
	T2c	3 (13.6%)
Pathological Gleason score *	N	19
	3 + 3	2 (10.5%)
	3 + 4	10 (52.6%)
	4 + 3	2 (10.5%)
	4 + 4	3 (15.8%)
	3 + 5	1 (5.3%)
	4 + 5	0 (0.0%)
	5 + 4	1 (5.3%)
Pathological T stage *	N	19
	T2a	3 (15.8%)
	T2c	8 (42.1%)
	T3a	7 (36.8%)
	T3b	1 (5.3%)
Jewett stage	N	22
	B	22 (100.0%)
	C-D2	0 (0.0%)

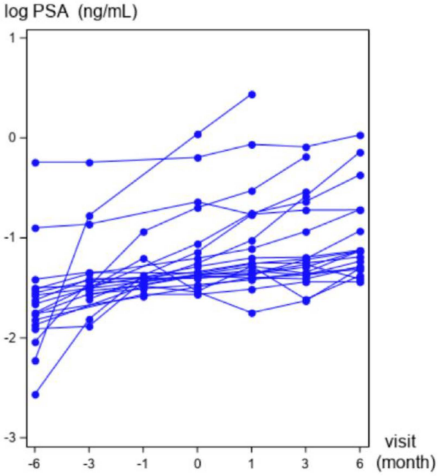
Table 1. *Cont.*

Factor		Group	N = 22
Time to biochemical recurrence after RP (year)		N	22
		Mean (SD)	4.2 (2.4)
Time to green propolis administration after RP (year)		N	22
		Mean (SD)	5.2 (2.7)
Performance status **		N	22
		0	22 (100.0%)
		1–4	0 (0.0%)
Past history		N	22
		no	8 (36.4%)
		yes	14 (63.6%)
History of previous CAM intake		N	22
		no	17 (77.3%)
		yes	5 (22.7%)
Blood test value		N	22
	WBC ($\times 10^9$/L)	Mean (SD)	5.1 (1.4)
	RBC ($\times 10^{12}$/L)	Mean (SD)	4.4 (0.4)
	Hb (g/dL)	Mean (SD)	13.9 (1.2)
	PLT ($\times 10^9$/L)	Mean (SD)	202.5 (41.0)
	LDH (U/l)	Mean (SD)	188.8 (32.9)
	ALP (U/l)	Mean (SD)	215.1 (70.0)
	GPT (U/l)	Mean (SD)	24.5 (13.5)
	GOT (U/l)	Mean (SD)	23.5 (7.7)
	Cre (mg/dL)	Mean (SD)	0.9 (0.2)
	BUN (mg/dL)	Mean (SD)	18.0 (3.3)
	Na (mEq/L)	Mean (SD)	141.0 (1.6)
	K (mEq/L)	Mean (SD)	4.3 (0.3)
	Cl (mEq/L)	Mean (SD)	105.1 (2.3)
	T-COL (mg/dL)	Mean (SD)	184.3 (20.6)
	TG (mg/dL)	Mean (SD)	118.1 (64.8)

Abbreviations: BMI, body mass index; PSA, prostate-specific antigen; RP, radical prostatectomy; CAM, complementary and alternative medicine; WBC, white blood cell count; RBC, red blood cell count; Hb, hemoglobin; Plt, platelet count; LDH, lactate dehydrogenase; ALP, alkaline phosphatase; GPT, glutamic pyruvic transaminase; GOT, glutamic oxaloacetic transaminase; Cre, creatinine; BUN, blood urea nitrogen; Na, sodium; K, potassium; Cl, chlorine; T-COL, total cholesterol; TG, triglyceride. * Three patients with neoadjuvant hormone therapy were excluded. ** Performance Status by ECOG (Common Toxicity Criteria, Version 2.0).

3.2. Therapeutic Effect of Brazilian Green Propolis

Figure 1 shows the actual alterations in log PSA before and after study entry. Propolis administration was discontinued in three patients due to a rapid rise in serum PSA values within six months. The PSA values in the two patients were decreased after baseline. In this study, the PSA response rate, the primary endpoint of this study, was 0%, and there was no apparent therapeutic effect of Brazilian green propolis. The estimates of PSA elevation rate from baseline at 1, 3, and 6 months was 112.1% (95% CI: 104.7, 119.4), 120.5% (95% CI: 107.1, 133.9), and 135.7% (95% CI: 112.7, 158.7), respectively (Figure 1). Of the twenty-two patients, four had PSA elevations over 25% after baseline. PSA progression occurred in two, one, and one patients at 1, 2, and 3 months, respectively.



Prostate-specific antigen values and elevation rates from baseline

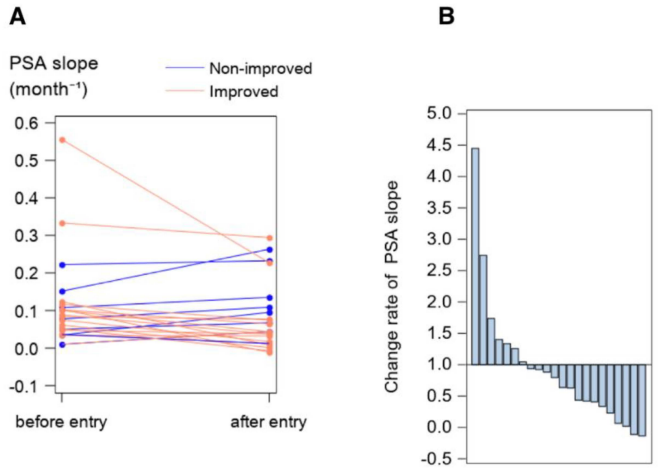
Time of observation	N	PSA value Mean (SD) (ng/mL)	PSA elevation rate (95% CI) (%)
Baseline	22	0.34 (0.21)	
At 1 month	22	0.40 (0.31)	112.1 (104.7, 119.4)
At 3 months	21	0.38 (0.20)	120.5 (107.1, 133.9)
At 6 months	19	0.40 (0.22)	135.7 (112.7, 158.7)

Abbreviations: PSA, prostate-specific antigen; CI, confidence interval

Figure 1. Prostate-specific antigen levels before and after administration of Brazilian green propolis.

3.3. Impacts on the PSA Slope

The mean PSA slopes before and after baseline were 0.12 month^{-1} and 0.08 month^{-1}, respectively (Figure 2). Figure 2A shows the actual alteration in the PSA slope before and after baseline. The PSA slopes in all patients before baseline were positive. Of the 22 patients, 15 had a PSA slope ratio <1, and the PSA slope response rate was 68.2% (95% CI: 45.1, 86.1) (Figure 2B).



The secondary end-point; prostate-specific antigen slope response rate

Time of observation	*N*	PSA-DT Mean (SD) (month)	PSA slope Mean (SD) (month^{-1})	Number of the cases with decreased PSA slope	PSA slope response rate (95% CI) (%)
Before entry	22	17.47 (20.27)	0.12 (0.12)	15	68.2 (45.1, 86.1)
After entry	22	53.32 (197.67)	0.08 (0.09)		

Abbreviations: PSA-DT, PSA (prostate-specific antigen) doubling time
95% confidence interval (CI) was calculated using the Clopper–Pearson method.

Figure 2. The secondary endpoint; prostate-specific antigen (PSA) slope kinetics. (**A**) Effect of Brazilian green propolis administration on PSA slope dynamics. **(B)** Alterations in the PSA slope ratio. The PSA slope ratio was calculated by dividing the PSA slope after baseline by that before baseline.

3.4. Characteristics of the Two Groups: PSA Slope Ratio < 1 Group and PSA Slope Ratio ≥ 1 Group

Table 2 shows the characteristics of patients with PSA slope ratios < 1 and ≥1. The patients in the PSA slope ratio ≥ 1 group had a higher clinical stage than those in the PSA slope ratio < 1 group (p = 0.01). The PSA value at diagnosis and baseline, pathological Gleason score, pathological T stage, and time to BCR were similar between the two groups. The mean AR staining score in the PSA slope ratio ≥ 1 group was 4.2, which was higher than that in the PSA slope ratio <1 group (1.7, p = 0.03). Additionally, the mean AKR1C3 staining score was 1.8 and 4.0 in patients with PSA levels <1 and ≥1, respectively (p = 0.07). All six patients with a score of 0 on AKR1C3, AR, and ERG staining had a PSA slope ratio < 1.

Table 2. Characteristics of the two groups: prostrate-specific antigen slope ratio < 1 group and prostate-specific antigen slope ratio ≥ 1 group.

Factor		**PSA Slope Ratio < 1 N = 15**	**PSA Slope Ratio ≥ 1 N = 7**	***p*-Value** [§]
Age (year old)	N	15	7	
	Mean (SD)	69.7 (6.2)	73.9 (3.7)	0.12
BMI (kg/m^2)	N	15	7	
	Mean (SD)	23.0 (2.5)	22.2 (2.8)	0.49
Initial PSA (ng/mL)	N	15	7	
	Mean (SD)	8.5 (4.8)	11.2 (5.1)	0.25
PSA level at study entry (ng/mL)	N	15	7	
	Mean (SD)	0.3 (0.2)	0.4 (0.2)	0.45
Biopsy Gleason score	N	15	7	
	3 + 3	5 (33.3%)	0 (0.0%)	0.69 [§§]
	3 + 4	2 (13.3%)	4 (57.1%)	
	4 + 3	3 (20.0%)	0 (0.0%)	
	4 + 4	4 (26.7%)	2 (28.6%)	
	4 + 5	0 (0.0%)	1 (14.3%)	
	5 + 4	1 (6.7%)	0 (0.0%)	
Clinical T stage	N	15	7	
	T1c	7 (46.7%)	0 (0.0%)	0.01 [§§]
	T2a	7 (46.7%)	3 (42.9%)	
	T2b	0 (0.0%)	2 (28.6%)	
	T2c	1 (6.7%)	2 (28.6%)	
Pathological Gleason score *	N	13	6	
	3 + 3	1 (7.7%)	1 (16.7%)	1.00 [§§]
	3 + 4	7 (53.8%)	3 (50.0%)	
	4 + 3	2 (15.4%)	0 (0.0%)	
	4 + 4	2 (15.4%)	1 (16.7%)	
	3 + 5	0 (0.0%)	1 (16.7%)	
	5 + 4	1 (7.7%)	0 (0.0%)	
Pathological T stage *	N	13	6	
	T2a	2 (15.4%)	1 (16.7%)	0.67 [§§]
	T2c	7 (53.8%)	1 (16.7%)	
	T3a	3 (23.1%)	4 (66.7%)	
	T3b	1 (7.7%)	0 (0.0%)	
Jewett stage	N	15	7	
	B	15 (100.0%)	7 (100.0%)	-
	C-D2	0 (0.0%)	0 (0.0%)	
Time to biochemical recurrence after RP (year)	N	15	7	
	Mean (SD)	3.5 (2.0)	5.8 (2.5)	0.03

Table 2. *Cont.*

Factor			PSA Slope Ratio < 1 N = 15	PSA Slope Ratio ≥ 1 N = 7	*p*-Value §
Time to green propolis administration after RP (year)		N	15	7	
		Mean (SD)	4.4 (2.2)	6.9 (3.1)	0.03
Performance status **		N	15	7	
		0	15 (100.0%)	7 (100.0%)	-
		1–4	0 (0.0%)	0 (0.0%)	
Past history		N	15	7	
		no	6 (40.0%)	2 (28.6%)	1.00
		yes	9 (60.0%)	5 (71.4%)	
History of previous CAM intake		N	15	7	
		no	12 (80.0%)	5 (71.4%)	1.00
		yes	3 (20.0%)	2 (28.6%)	
Immunohistochemistry *		N	13	6	
	AKR1C3	Mean (SD)	1.8 (2.6)	4.0 (1.3)	0.07
	ERG	Mean (SD)	0.7 (1.8)	2.2 (3.4)	0.22
	AR	Mean (SD)	1.7 (2.0)	4.2 (2.3)	0.03
Blood test value		N	15	7	
	WBC ($\times 10^9$/L)	Mean (SD)	5(1.5)	5.3(1.2)	0.68
	RBC ($\times 10^{12}$/L)	Mean (SD)	4.5 (0.4)	4.3 (0.6)	0.52
	Hb (g/dL)	Mean (SD)	14.0 (1.1)	13.5 (1.5)	0.40
	PLT ($\times 10^9$/L)	Mean (SD)	204.2 (40.5)	198.9(45.2)	0.78
	LDH (U/l)	Mean (SD)	187.7 (32.0)	191.1(37.3)	0.82
	ALP (U/l)	Mean (SD)	219.5 (82.7)	205.7 (32.0)	0.68
	GPT (U/l)	Mean (SD)	26.1(15.3)	20.9 (8.3)	0.41
	GOT (U/l)	Mean (SD)	24.3 (8.2)	22 (7.1)	0.54
	Cre (mg/dL)	Mean (SD)	0.9 (0.2)	0.8 (0.1)	0.34
	BUN (mg/dL)	Mean (SD)	17.9 (3.5)	18.3(3.1)	0.79
	Na (mEq/L)	Mean (SD)	140.8 (1.7)	141.4(1.4)	0.41
	K (mEq/L)	Mean (SD)	4.3 (0.3)	4.1 (0.3)	0.21
	Cl (mEq/L)	Mean (SD)	105.2 (2.5)	105 (1.9)	0.85
	T-COL (mg/dL)	Mean (SD)	181.3 (20.0)	190.7 (21.9)	0.33
	TG (mg/dL)	Mean (SD)	119.1 (65.0)	115.9(69.4)	0.92

Abbreviations: BMI, body mass index; PSA, prostate-specific antigen; RP, radical prostatectomy; CAM, complementary and alternative medicine; AKR1C3, aldo-keto reductase 1C3; ERG, Ets-Related Gene; AR, androgen receptor; WBC, white blood cell count; RBC, red blood cell count; Hb, hemoglobin; Plt, platelet count; LDH, lactate dehydrogenase; ALP, alkaline phosphatase; GPT, glutamic pyruvic transaminase; GOT, glutamic oxaloacetic transaminase; Cre, creatinine; BUN, blood urea nitrogen; Na, sodium; K, potassium; Cl, chlorine; T-COL, total cholesterol; TG, triglyceride. * Three patients with neoadjuvant hormone therapy were excluded. ** Performance Status by ECOG (Common Toxicity Criteria, Version 2.0). § The continuous data were compared with the mean values using Student's *t*-test. Of the categorical data, §§ was compared by the Cochran-Armitage test and the rest by Fisher's exact test.

3.5. Alteration of Testosterone Values

The testosterone response rates at 1, 3, and 6 months were 14.3% (95% CI: 3.0, 36.3), 4.8% (95% CI: 0.1, 23.8), and 31.3% (95% CI: 11.0, 58.7), respectively (Table 3). The testosterone response with Brazilian green propolis was limited.

Table 3. The secondary endpoint; testosterone response rate.

Time of Observation	N	Teststerone Level Mean (SD) (ng/dL)	N	Change Rate of Teststerone Level (95% CI) (%)	Number of the Cases with ≥20% Decreased Teststerone Level	Testosterone Response Rate (95% CI) (%)
Baseline	21	414.12 (169.62)				
At 1 month	21	439.40 (167.01)	21	108.8 (99.4, 118.3)	3	14.3 (3.0, 36.3)
At 3 months	21	442.90 (173.14)	21	108.7 (99.8, 117.6)	1	4.8 (0.1, 23.8)
At 6 months	16	385.53 (151.01)	16	101.9 (86.1, 117.7)	5	31.3 (11.0, 58.7)

95% confidence interval (CI) was calculated using the Clopper–Pearson method.

3.6. Effect on PRO

The changes in the FACT-P score over time using a linear mixed-effects model are shown in Table 4 and Figure 3. Each of the least-squares mean difference of the total score from baseline were −1.14 (95% CI: −8.01, 5.74), 1.49 (95% CI: −5.5, 8.49), and 4.94 (95% CI: −2.5, 12.37), respectively (Table 4, Figure 3). The least-squares mean of the emotional domain tended to continue to rise. Each of the least-squares mean differences in the emotional well-being domain scores from baseline were 0.30 (95% CI: −0.78, 1.38), 0.48 (95% CI: −0.64, 1.59), and 0.70 (95% CI: −0.48, 1.88) (Table 4, Figure 3).

Table 4. Changes from baseline of the Functional Assessment of Cancer Therapy-Prostate (FACT-P) score.

Domain		Score Range	Time of Observation	*N*	Least-Squares Mean	Standard Error	Least-Squares Mean Difference from Baseline	*p*-Value
Total score		0–156	Baseline	19	122.13	3.52		
			At 1 month	22	120.99	3.52	−1.14 (−8.01, 5.74)	0.74
			At 3 months	20	123.62	3.58	1.49 (−5.50, 8.49)	0.67
			At 6 months	17	127.07	3.80	4.94 (−2.50, 12.37)	0.19
	G (general)	0-108	Baseline	20	85.02	2.76		
			At 1 month	22	85.84	2.76	0.82 (−5.19, 6.82)	0.79
			At 3 months	20	88.77	2.87	3.75 (−2.46, 9.96)	0.23
			At 6 months	17	92.12	3.08	7.10 (0.49, 13.7)	0.04
	Physical well-being	0–28	Baseline	20	26.60	0.26		
			At 1 month	22	25.98	0.26	−0.63 (−1.29, 0.04)	0.07
			At 3 months	20	26.32	0.27	−0.28 (−0.97, 0.41)	0.42
			At 6 months	17	26.32	0.30	−0.28 (−1.01, 0.45)	0.44
	Social/family well-being	0–32	Baseline	20	19.63	1.58		
			At 1 month	22	18.81	1.58	−0.82 (−4.51, 2.88)	0.66
			At 3 months	20	21.37	1.65	1.75 (−2.08, 5.57)	0.36
			At 6 months	17	24.06	1.78	4.43 (0.37, 8.48)	0.03
	Social/family well-being (Answer is optional)	(0–4)	Baseline	15	1.05	0.28		
			At 1 month	17	0.92	0.30	−0.13 (−0.85, 0.58)	0.70
			At 3 months	14	0.95	0.31	−0.10 (−0.83, 0.63)	0.78
			At 6 months	11	1.32	0.34	0.26 (−0.52, 1.05)	0.50
	Emotional well-being	0–24	Baseline	20	18.71	0.46		
			At 1 month	22	19.01	0.46	0.30 (−0.78, 1.38)	0.58
			At 3 months	20	19.18	0.48	0.48 (−0.64, 1.59)	0.40
			At 6 months	17	19.41	0.52	0.70 (−0.48, 1.88)	0.24
	Functional well-being	0–28	Baseline	20	20.02	1.22		
			At 1 month	22	21.98	1.22	1.96 (−0.92, 4.83)	0.18
			At 3 months	20	21.91	1.27	1.89 (−1.08, 4.86)	0.21
			At 6 months	17	22.33	1.38	2.32 (−0.84, 5.47)	0.15

Table 4. *Cont.*

Domain		Score Range	Time of Observation	*N*	Least-Squares Mean	Standard Error	Least-Squares Mean Difference from Baseline	*p*-Value
	P (prostate)	0–48	Baseline	19	35.74	1.08		
			At 1 month	22	35.85	1.08	0.11 (−2.01, 2.23)	0.92
			At 3 months	20	34.37	1.09	−1.36 (−3.52, 0.79)	0.21
			At 6 months	17	34.52	1.16	−1.22 (−3.51, 1.08)	0.29

FACT-P score was evaluated using a linear mixed-effects model. The model included baseline and time predictors as fixed effects and a random intercept.

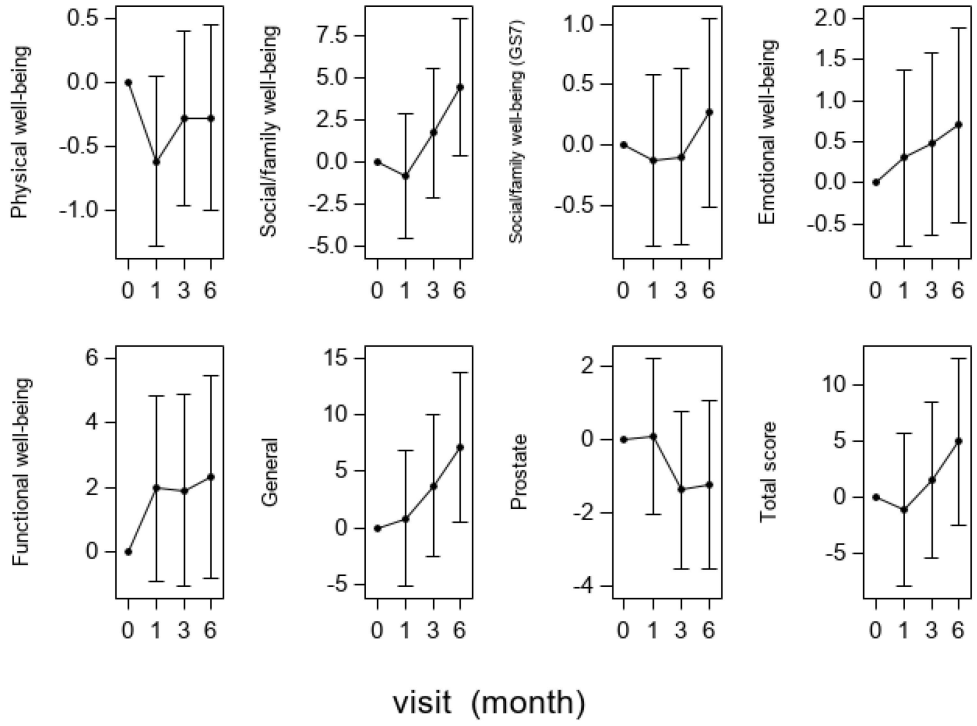


Figure 3. Least-squares mean differences between the baseline and time points for each domain in the Functional Assessment of Cancer Therapy-Prostate (FACT-P) questionnaire score. FACT-P score was evaluated using a linear mixed-effects model. The model included baseline and time predictors as fixed effects and a random intercept.

3.7. Safety Profiles of Brazilian Green Propolis

Adverse effects were observed in six patients: four patients had diarrhea (Grade 1), one patient had anorexia (Grade 1), and one patient had constipation (Grade 2). There were no serious adverse events leading to the discontinuation of the study (Table 5). Consumption of Brazilian green propolis by patients without allergies was considered safe.

Table 5. Safety profiles of Brazilian green propolis.

Category	Grade1	Grade2	Grade3	Grade4	Total
	N	N	N	N	N (%)
Diarrhea	4				4 (18.2)
Constipation		1			1 (4.5)
Anorexia	1				1 (4.5)

4. Discussion

While overall cancer control rates are mostly acceptable for clinically localized PCa after RP, and improved outcome of RP using novel imaging tools such as prostate specific

membrane antigen (PSMA) positron emission tomography (PET)/CT and indocyanine green (ICG) guidance might be expected, 20–30% of patients experience a recurrence that initially presents as elevated serum PSA without clinical or radiographic metastases [2,27–29]. However, not all patients with BCR develop disease progression and metastases, and the clinical course of the patients is highly variable. The European Association of Urology and three other related associations recommend using a novel BCR classification system that stratifies patients with BCR into low-risk (PSA-DT > 1 year and pathological Gleason score [pGS] < 8) and high-risk (PSA-DT $\leq$ 1 year or pGS 8–10) [30]. An external validation of this risk classification shows that the five-year PCa-specific mortality-free survival rates were 99.7% for the low-risk group and 86.7% for the high-risk group [31]. Furthermore, SR therapy had some impact on prostate cancer specific mortality for patients with PSA-DT < 6 months but not for those with PSA-DT $\geq$ 6 months [32]. Therefore, for patients with a PSA-DT $\geq$ 6 months, delaying the initiation of SR therapy after BCR might be an acceptable choice, whereas those with high-risk factors are recommended to undergo SR therapy before PSA levels rise to 0.5 ng/mL [4–6,30]. This presents an opportunity to administer CAM to patients who had developed BCR post-RP till their PSA levels rise to 0.5 ng/mL.

A PCa patient, who visited our out-patient clinic and developed a recurrence after RP, underwent SR, and received intermittent bicalutamide treatment, showed a marked reduction in his serum PSA levels after ingesting Brazilian green propolis during the period when he was not on bicalutamide treatment. Importantly, he ingested ten times more than the usual dose of Brazilian green propolis. Additionally, the AKR1C3 level explored by immunohistochemistry in his RP specimen was positive (data not shown). Therefore, we set a primary endpoint to elucidate the degree of anticancer effects of Brazilian green propolis in this clinical trial.

We found no anticancer effect of Brazilian green propolis against BCR after RP since no objective response was obtained in the serum PSA levels. However, we showed that the PSA slope was decreased after propolis administration. Although we did not assign placebo-control groups and the low PSA levels in our study sample may preclude us from calculating reliable PSA-DT [33], we included PSA values before and after 6 months of propolis administration, and the PSA levels in most patients increased steadily compared with before baseline. Therefore, the PSA-DT and PSA slopes calculated in our study could reliably reflect the disease status [34], and this supplement may have a mild impact on PCa. Our results corroborate those of Endo et al., who reported that artepillin C, a cinnamic acid derivative in Brazilian green propolis, induced apoptosis in PCa CWR22Rv1 cells [35]. Additionally, baccharin, another component of Brazilian green propolis, is a selective inhibitor of AKR1C3 and therefore might effectively suppress PCa. Furthermore, baccharin is chemically unstable and hydrolyzes into drupanin, which possesses attenuated AKR1C3 inhibitory activities [36]. The elevated AKR1C3 expression in the RP specimen of our pilot patient led us to theorize that the excess dosage of propolis he had ingested could have affected his PCa cells, causing a notable reduction in his PSA levels. However, based on our study, patients with low AKR1C3 expression in prostate specimens showed a decrease in the PSA slope after propolis administration. The propolis dosage in our study might not have been sufficient to block the AKR1C3 function in cancer cells because of its high expression level. Therefore, patients with lower AR and AKR1C3 expression might have had a PCa with less aggressive biology and might have received some benefit from propolis that resulted in a PSA slope <1 group [18,37]. Furthermore, since the AKR1C3 expression level in PCa cells collected from the initial RP specimen and the post-RP recurrent lesion might differ, the association between AKR1C3 expression and effectiveness of propolis remains unknown.

We also evaluated the safety profile of Brazilian green propolis in patients with BCR after RP. Most patients did not exhibit any adverse events, except for a few who had mild bowel symptoms (diarrhea or constipation). There was a slight influence of interven-

tion compounds on serum testosterone levels; however, the levels remained within the normal range.

The quality of life (QOL) for those who received propolis in our trial was also investigated using the FACT-P questionnaire. Although the total scores showed an improvement in QOL from baseline, this change was not statistically significant [38]. Most of the subdomains did not worsen during propolis administration, showing that administering propolis may not negatively impact the QOL in patients with BCR after RP.

There are several limitations to our study. First, we conducted the study as a single-arm, without a placebo-control group. However, the characteristic odor of Brazilian green propolis capsules makes them difficult to use in a placebo-control setting. Since we had difficulty setting the external control, we evaluated the PSA slope as the internal control. The PSA slope is the rate of PSA increase per unit time, and it was assumed that comparing the PSA slope before and after propolis administration in a single patient approximated the effect on PSA kinetics after propolis administration. Second, the Brazilian green propolis we used contained many unique compounds, which makes it difficult to identify active chemical agents. Third, propolis dosage for inhibiting prostate cancer cell growth after BCR is still unknown, and the dosage assigned in our study may be insufficient for cancer control. Moreover, it is difficult to strictly control inter- and intra-lot variability of the Brazilian green propolis because of natural product extraction, although the product we used was manufactured and quality-controlled by the same company. Therefore, we do not know whether similar results will be obtained against BCR after RP on administrating a different brand of Brazilian green propolis product. Fourth, our sample size was too small to draw a definitive conclusion. Despite the limitations listed above, we believe that our clinical trial provides important information regarding the use of Brazilian green propolis against BCR after RP.

5. Conclusions

We administered Brazilian green propolis for the treatment of biochemical recurrence. No significant anticancer response was observed for Brazilian green propolis; however, 68% showed a decreased PSA slope. Brazilian green propolis may be safely consumed by patients without any related allergies.

Author Contributions: T.G., S.T., R.U., S.M., O.O. and T.I. were involved in conception and design. T.G., H.K., T.Y. (Takayuki Yoshino), A.S., S.A., T.K., T.Y. (Toshinari Yamasaki), O.O. and T.I. were involved in acquisition of data. T.G., H.K. and M.F. were involved in analysis and interpretation of data. T.G., H.K., Y.H., R.U. and T.I. were involved in drafting of the manuscript. Y.H., R.U. and S.M. were involved in statistical analysis. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki and approved by the Institutional Review Board and ethics committees of Kyoto University Graduate School of Medicine (institutional review board number: YC1196-2) and registered in the UMIN Clinical Trials Registry (registration number: UMIN 000023451).

Informed Consent Statement: Written informed consent was obtained from all subjects involved in the study.

Data Availability Statement: Not applicable. Enrollment and data management were performed in an independent data center at the Translational Research Center, Kyoto University Hospital.

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Abbreviations

AKR1C3	Aldo-keto reductase 1C3
AR	Androgen receptor
BCR	Biochemical recurrence
CAM	Complementary and alternative medicine
CI	Confidence interval
ERG	Ets-Related Gene
FACT-P	Functional Assessment of Cancer Therapy-Prostate
PCa	Prostate cancer
PG	prostaglandin
pGS	Pathological Gleason score
PRO	Patient-Reported Outcomes
PSA	Prostate-specific antigen
PSA-DT	PSA doubling time
QOL	Quality of life
RP	Radical prostatectomy
SR	Salvage radiation

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