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Impact of Solarplast® on Oxidative Stress, Immune Function, and Skin Appearance in Active Smokers and Non-Smokers

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Abstract

Background: Dietary antioxidants are widely used to reduce oxidative stress and enhance immune system function. Smoking is a known generator of reactive oxygen species, leading to oxidative stress, which can adversely affect general health and skin health. **Methods:** We completed a randomized, placebo-controlled double-blind clinical trial to investigate the effects of Solarplast®, an enzyme treated spinach supplement, on oxidative stress, immune function, and skin health, in a sample of male and female smokers and non-smokers. Fifty-nine participants were randomly assigned to 60-day daily ingestion of Solarplast® (100 mg) or placebo to determine the effects on white blood cell counts and distribution, protein oxidation, lipid peroxidation, and ex vivo cytokine concentration (TNF-α, IL-6, IL-10, and IL-1β) with and without lipopolysaccharide (LPS) stimulation. Normalized cytokine levels were determined by dividing the LPS-stimulated levels by the non-stimulated levels. Skin health was assessed using a Pear 3D Plus Skin Analysis system and questionnaires determined subjects' perceived health. Change scores from baseline were used for comparing treatments and results between smokers and non-smokers. **Results:** Solarplast® was well-tolerated, with no adverse events noted. Treatment did not result in a statistically significant reduction for oxidative stress markers of Advanced Oxidation Protein Products (AOPP) (13% reduction) or malondialdehyde (MDA) (15% reduction) from baseline in smokers. Treatment reduced normalized ex vivo TNF-α levels (58%) from baseline, but this finding did not reach statistical significance due to the high variability across subjects. Solarplast® significantly reduced *ex vivo* normalized IL-1β more than 30% at both day 30 ($p = 0.008$) and day 60 ($p = 0.026$) as compared to placebo, with a more pronounced effect in the non-smokers (43% and 46% reduction at day 30 and day 60, respectively) as compared to the smokers (31% and 25% reduction at day 30 and day 60, respectively). Values for measure-

ments of skin appearance resulted in higher moisture levels at day 60 for the Solarplast® group compared to placebo, regardless of smoking status. **Conclusion:** This exploratory study suggests that Solarplast®, a novel spinach supplement appears safe and well-tolerated for human consumption and can reduce the normalized cytokine response *ex vivo*—specifically IL-1β. As there were improvements to other outcomes that did not reach statistical significance, further work involving at longer intervention time and varied dosing regimens is warranted to more fully realize the potential benefits of Solarplast® on health-specific parameters.

Keywords

Solarplast®, Oxidative Stress, Immune Function

1. Introduction

Reactive oxygen species (ROS), such as singlet oxygen (·O), superoxide radical ($O_2^{\bullet-}$), and hydroxyl radical (·OH) are by-products of normal aerobic metabolism in living cells that can be exacerbated by environmental stressors [1]. When ROS are generated in quantities that exceed the body's innate antioxidant defense mechanisms, oxidative stress occurs, contributing to the development of numerous diseases including diabetes, cancer, and cardiovascular diseases [2].

Cigarette smoking is a major source of ROS, exposing smokers to over 10^{15} free radicals per puff in the gas phase and over 10^{17} free radicals per gram in the tar phase [3] [4]. This direct exposure represents only a fraction of the total oxidative stress experienced, as cigarette smoke contributes to further endogenous ROS production mediated through inflammatory and immune processes [5]. Furthermore, evidence suggests that ROS formation due to cigarette smoking may contribute to premature skin aging [6]. Similarly, vaping has been found to increase oxidative stress markers [7] in blood and generate ROS species [8] [9], with one single 30-minute vaping session shown to cause oxidative stress levels to increase 2 - 4 times in non-smokers [10], due to the inhalation of toxic compounds present in the vaping solution [10]-[12].

Oxidative stress can also lead to the production proinflammatory cytokines such as IL-1β and TNF-α *in vivo* [2]. Similarly, in many *in vitro* models, lipopolysaccharide (LPS) can be used as a proxy to induce ROS and proinflammatory cytokines [13]. Both oxidative stress and inflammation are linked with an array of neurological disorders [14], and recent evidence suggests the antioxidants present within spinach, such as carotenoids [15], may provide some protection against LPS and oxidative stress [16] [17], and thus may alleviate some of the negative effects on mood and skin health associated with oxidative stressors such as smoking.

Spinach is rich in select vitamins and minerals, such as vitamin A and lutein—a carotenoid that acts as a natural antioxidant, supporting the body's natural antioxidant defensive mechanisms [16]. It contains photosynthetic complexes that

contribute to scavenging free radicals and attenuate ensuing damage from ROS.

Solarplast® is an enzyme treated spinach supplement. Recent *in vitro* evidence indicates that Solarplast® may have potential antioxidant activity [17]. This exploratory study aimed to evaluate the effects of Solarplast® on oxidative stress, immune function, skin appearance, and related measures in both smokers and non-smokers. We hypothesized that Solarplast® would reduce oxidative stress biomarkers, as well as favorably impact immune parameters and measures of skin health, in both smokers and non-smokers, with a greater impact observed in the smoking participants.

2. Materials and Methods

2.1. Study Participants

The trial was conducted according to the Declaration of Helsinki of 1975 and ICH-Good Clinical Practice (GCP) guidelines, revised in 2013; approved by the University Institutional Review Board for Human Subjects Research (protocol #PRO-FY2021-416) at the University of Memphis and registered on clinicaltrials.gov (NCT05162820). The trial was conducted as a randomized, placebo-controlled double-blind clinical trial, between October 2021 and June 2023 at a single site in Memphis, TN, USA. For this exploratory pilot study, no power analysis was performed but a convenience sample of 14 men and 36 women (completers), based on prior unpublished pilot work, were recruited and completed all phases of this study, including 16 active smokers and 34 non-smokers. See Table 1 for subject descriptive characteristics.

Table 1. Baseline subject characteristics by condition [mean (SD) or n (%)].

Characteristics	Treatments		
	Placebo (n = 24)	Solarplast® (n = 26)	Total (n = 50)
Smoking Status, n (%)			
Smoker	7 (29.2)	9 (34.6)	16 (32.0)
Nonsmoker	17 (70.8)	17 (65.4)	34 (68.0)
Age (yrs)	43.9 (10.2)	45.3 (10.5)	44.6 (10.3)
Gender, n (%)			
Male	8 (33.3)	6 (23.1)	14 (28.0)
Female	16 (66.7)	20 (76.9)	36 (72.0)
Height (cm)	167.3 (9.3)	167.0 (9.8)	167.2 (9.4)
Weight (kg)	77.6 (18.2)	80.8 (18.4)	79.3 (18.2)
Body Mass Index (kg/m^2)	27.6 (5.6)	29.0 (6.2)	28.3 (5.9)
Waist (cm)	89.3 (15.0)	89.6 (16.1)	89.5 (15.4)
Hip (cm)	104.5 (12.0)	106.0 (11.4)	105.3 (11.6)
Waist/Hip	0.85 (0.09)	0.84 (0.09)	0.85 (0.09)
Systolic Blood Pressure (mmHg)	116.2 (10.2)	116.0 (15.5)	116.1 (13.1)
Diastolic Blood Pressure (mmHg)	76.1 (8.9)	76.7 (11.3)	76.4 (10.1)
Heart Rate (beats/min)	72.7 (10.4)	75.3 (8.4)	74.0 (9.4)

Subjects were required to be 30 - 60 years old, with a body mass index (BMI) between 18 - 39.9 kg/m^2 (not morbidly obese). Smokers were initially required to smoke a minimum of 5 cigarettes daily and experience occasional mild skin irritations such as rash, dryness, redness, or itching. Due to challenges with subject recruitment for smokers, the criteria were broadened to include individuals who smoke cigarettes or vape regularly, for the past 12 months, and experience skin irritations. Non-smokers could not have used tobacco products or any smoked product (e.g., marijuana, cigar, pipe) in the past 12 months and could not be exposed to regular second-hand smoke (e.g., live with or work in close proximity to a smoker). Additional exclusion criteria included alcohol consumption within 48 hours of testing, caffeine consumption within 24 hours of testing, active infections or illnesses, pregnancy, and the use of medications or supplements that might impact the study outcomes including immune function or antioxidant nutritional supplements, over the counter medications such as vitamin-C, elderberry, ginger, turmeric, quercetin, supplements with "Immun" or have immunity on their label, immunosuppressives: prednisolone, azathioprine, mycophenolate mofetil, monoclonal antibodies—of which there are many ending in "mab", such as bevacizumab, rituximab and trastuzumab, anti-TNF drugs such as etanercept, infliximab, adalimumab, certolizumab and golimumab, methotrexate, ciclosporin, tacrolimus, sirolimus, cyclophosphamide, leflunomide, immunotherapies: immune checkpoint inhibitors, adoptive cell therapies, monoclonal antibodies, oncolytic virus therapy, immune system modulators, etc.

2.2. Informed Consent and Screening

All participants were informed of the study procedures, risks, and benefits through verbal and written forms. During the screening visit, participants completed health history, medication and dietary supplement usage, and physical activity questionnaires. Measurements of heart rate, blood pressure, height, weight, waist, and hip circumference were taken. Women self-administered a pregnancy test. Eligible subjects were then assigned a subject number for privacy and scheduled for their initial testing visit. Menstruating women began the study within the first 5 days of their menstrual cycle to control for hormonal fluctuations. Women with regular menstrual cycles would have reported for the day 30 and day 60 visit at a similar (although not identical) time of month.

2.3. Treatment

This exploratory study used a randomized, double-blind placebo-controlled design. Placebo and experimental capsules were produced according to Good Manufacturing Practices at Deerland Probiotics and Enzymes, Kennesaw, GA. The experimental capsules contained 100 mg of Solarplast® (enzymatically treated, non-genetically modified organism spinach extract), rice dextrin as needed, microcrystalline cellulose as needed and 1.2% medium chain triglycerides. Characterization of Solarplast's antioxidant capacity and phenolic component makeup have previ-

ously been reported [18]. The placebo capsules contained only rice dextrin, microcrystalline cellulose, and 1.2% medium chain triglycerides. Participants were instructed to take their assigned capsules daily with breakfast for 60 days.

2.4. Test Visit Procedures

Subjects reported to the lab in the morning following an overnight fast (>10 hours) where they filled out general health and well-being questionnaires (e.g., Warwick-Edinburgh Mental Wellbeing Scale [WEMWBS]) and an in-house skin health questionnaire in which subjects were instructed to indicate how they felt about their skin over the past week for each listed characteristic compared to how it normally is, using a discreet scale from 1 (very bad) to 5 (very good). These questionnaires were then filled out weekly during the study intervention. A skin analysis was performed using a Pear 3D Plus Skin Analysis system (DermaQuip, Marietta, GA), with a blood pressure and heart rate assessment being conducted after a 10-minute quiet rest. Thereafter, a blood sample was taken. This procedure was repeated at baseline (pre), 30 days, and 60 days.

2.4.1. Blood Collection

Single venipunctures were performed observing universal precautions to collect a 10 mL heparinized blood sample (BD Vacutainer REF367874, BD, Franklin Lakes, NJ) from subjects at baseline, 30 day, and 60 day test visits. These samples were analyzed for white blood cell counts and distribution (VetScan HM5, Abaxis, Union City, CA), protein oxidation (OxiSelect AOPP Assay STA-318, Cell Biolabs, Inc., San Diego, CA), lipid peroxidation biomarkers (Malondialdehyde Assay NWK-MDA01, Northwest Life Science Specialties, Vancouver, WA), and cytokines: TNF-α, IL-6, IL-10, and IL-1β (Milliplex HCYTA-60K-04 Human Cyto/Chem/GF Panel A, Millipore Sigma, Burlington, MA). For ex vivo cytokine analyses, blood samples were challenged with 250 ng/mL lipopolysaccharide (LPS) stimulation or control [19]. The blood borne variables were of the highest priority and viewed as our primary outcomes.

2.4.2. Pear 3D Plus Skin Analysis System by DermaQuip

The Pear 3D Plus system was used for assessing topographical, pigmentation and color, via photographical image analysis of the subjects' facial skin [20]. Photographic analyses like the Pear 3D Plus system have been developed for clinical use for monitoring skin health [21] and this tool was utilized in the present study for systematic analysis of each subject's skin for a variety of parameters including pores, wrinkles, elasticity, surface spots, UV spots, skin tone, moisture, and oil.

Images and analyses were performed by a trained investigator. Subjects placed their chin on the chin support before the system imaged their facial skin (~1 min) and then analyzed the images (<5 min) for the different skin variables. The analyses were used to monitor changes in predicted facial skin age for each individual skin variable at each visit.

2.4.3. Dietary Intake and Physical Activity

Subjects followed their usual activity patterns over the course of the study period but refrained from strenuous activity for the 48 hours preceding each test visit. Subjects also recorded their dietary intake on food logs for five days prior to each test visit, and data were analyzed using Food Processor Pro (Esha Research). Subjects were instructed to keep both their dietary intake and physical activity levels consistent over the 60-day study period.

2.5. Data Analysis

Descriptive statistics were used to outline baseline characteristics (means, standard deviations, frequencies and percentages). The assumptions of outliers and normal distribution of the data were inspected by using box-plots, normal probability plots, and the Shapiro-Wilk test of normality. Two-way mixed analyses of variance (ANOVA) were conducted to evaluate any differences in dietary intake variables between treatment groups at pre-treatment, 30 days, and 60 days. In addition, two-way ANOVAs were conducted to examine the effects of smoking status and treatment (Solarplast® vs. placebo) on biological variables, plasma cytokine variables, skin imaging, and self-reported feelings of health and well-being at baseline and two timepoints (e.g., timepoint 1 = 30 day minus baseline; timepoint 2 = 60 day minus baseline). Because the primary objective was to evaluate change from baseline, analyses were performed on change scores (30-day minus baseline and 60-day minus baseline), with baseline values incorporated into the outcome measure, rather than included as a separate covariate. If interaction effects were not significant, follow-up analyses were conducted by examining the main effects of each independent variable separately using one-way ANOVAs or independent t-tests, as appropriate. In cases where assumptions of normality were violated, non-parametric alternatives (e.g., Mann-Whitney U tests or Kruskal-Wallis tests) were employed. Statistical analyses were conducted using a per-protocol (complete-case) approach. Of the 59 participants randomized, 50 completed the study and contributed data to the primary analyses. Participants who withdrew before study completion were excluded from the analyses, and missing data resulting from participant withdrawal were not imputed. For individual outcome measures, analyses were conducted using all available data; therefore, sample sizes varied slightly across outcomes because of occasional missing measurements. All analyses were performed using the Statistical Package for the Social Sciences (SPSS for Windows, version 28; SPSS, Chicago, IL). All statistical tests were two-tailed with the significance level represented by $p < 0.05$.

3. Results

3.1. Lifestyle and Diet Factors

Fifty subjects completed the 60-day intervention. In addition to the individuals that completed the study, 32 additional individuals provided informed consent, but did not complete all aspects of the study for the following reasons: 21 for

scheduling issues; 2 due to illness unrelated to treatment; 1 due to problems with blood collection; 8 subjects missed visits and stopped communicating with the research staff. Detailed characteristics are in Table 1 and the CONSORT diagram is in Figure 1. Of the 16 smokers that completed the study, 62.5% reported solely smoking traditional cigarettes, while the remainder used a combination or solely vaped. All smokers regardless of use type were analyzed together. Compliance was determined via capsule counts and found to be similar between treatments with 94.6% for Solarplast® and 93.5% for placebo.

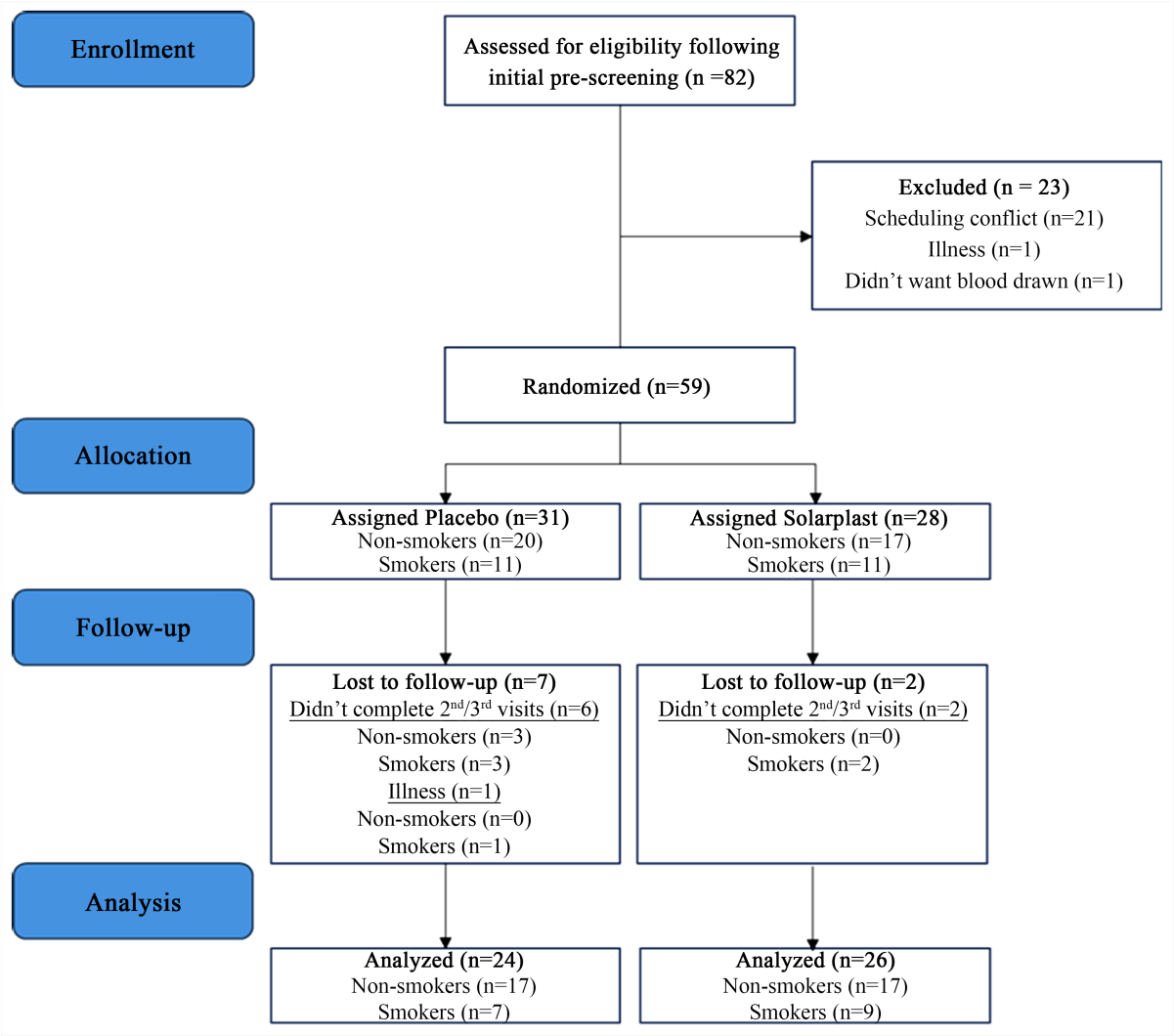

Figure 1. CONSORT Diagram specific to the impact of Solarplast® on oxidative stress, immune function, and skin appearance in active smokers and non-smokers.

There were no statistically significant changes to subjects' self-reported behavior related to exercise, sleep, alcohol consumption, or water consumption throughout the course of the study, (Table 2; $p > 0.05$). Additionally, subjects' nutrition remained consistent (Table 3; $p > 0.05$), suggesting these effects should not have influenced the outcomes.

Table 2. Psychosocial measures of participants pre-treatment, 30 day, and 60 day and participants' significance of change outcomes.

Measures	Placebo Mean ± SD		Solarplast® Mean ± SD			Outcomes Across Time p-value		
	Smoker n = 7	Non-Smoker n = 17	Smoker n = 9	Non-Smoker n = 17		Interaction	Treatment	Smoking Status[4]
WEMWBS								
Pre	55.67 ± 6.31	54.53 ± 7.41	51.33 ± 5.98	52.47 ± 5.68				
30 day	54.67 ± 8.14	55.60 ± 9.66	49.11 ± 9.88	50.00 ± 9.03	T1[3]	0.533	0.226	0.615
60 day	59.33 ± 5.39	57.20 ± 7.19	48.33 ± 10.50	50.88 ± 9.18	T2	0.509	0.003*	0.879
Exercise (days/wk)								
Pre	1.43 ± 1.40	3.06 ± 1.57	2.89 ± 2.26	3.47 ± 2.15				
30 day	1.57 ± 1.27	2.50 ± 1.75	2.00 ± 1.87	3.24 ± 2.02	T1	0.137	0.404	0.983
60 day	1.86 ± 1.07	2.88 ± 1.86	2.44 ± 2.56	3.71 ± 2.20	T2	0.159	0.609	0.934
Sleep (hours/night)								
Pre	7.23 ± 1.11	6.91 ± 1.00	7.25 ± 0.79	7.18 ± 0.71				
30 day	6.79 ± 1.22	6.77 ± 0.95	6.83 ± 0.87	7.39 ± 1.17	T1	0.622	0.423	0.068
60 day	7.00 ± 0.82	6.86 ± 1.36	7.11 ± 1.67	7.32 ± 0.77	T2	0.961	0.598	0.961
Water (cups/day)								
Pre	7.93 ± 2.65	8.27 ± 4.61	5.69 ± 2.79	7.51 ± 2.77				
30 day	7.57 ± 1.99	8.06 ± 4.67	6.67 ± 3.20	7.41 ± 2.45	T1	0.567	0.502	0.666
60 day	7.29 ± 1.50	6.88 ± 3.04	6.89 ± 3.52	8.68 ± 4.10	T2	0.726	0.065	0.704
Alcohol (servings/day)								
Pre	0.36 ± 0.48	0.47 ± 0.80	1.31 ± 1.90	0.93 ± 1.27				
30 day	0.21 ± 0.39	0.41 ± 0.58	0.68 ± 1.11	1.41 ± 2.18	T1	0.161	0.937	0.106
60 day	0.21 ± 0.39	0.36 ± 0.54	0.79 ± 1.08	1.14 ± 1.30	T2	0.187	0.918	0.147
Sleeping Quality								
Pre	2.19 ± 0.60	2.35 ± 0.80	2.67 ± 1.09	2.71 ± 0.73				
30 day	2.19 ± 0.69	2.10 ± 0.94	2.74 ± 1.15	2.55 ± 0.80	T1	0.954	0.676	0.242
60 day	1.57 ± 0.37	1.98 ± 0.57	2.56 ± 1.00	2.41 ± 0.85	T2	0.346	0.200	0.889
Mental Health[1]								
Pre	1.60 ± 0.24	2.05 ± 0.62	2.38 ± 0.41	2.23 ± 0.45				
30 day	1.68 ± 0.55	1.77 ± 0.66	2.38 ± 0.73	2.13 ± 0.50	T1	0.321	0.726	0.102
60 day	1.57 ± 0.41	1.60 ± 0.44	2.26 ± 0.87	2.03 ± 0.70	T2	0.641	0.209	0.443
Physical Health[2]								
Pre	1.89 ± 0.38	1.48 ± 0.28	1.77 ± 0.31	1.75 ± 0.27				
30 day	1.95 ± 0.20	1.41 ± 0.23	1.74 ± 0.31	1.61 ± 0.44	T1	0.342	0.150	0.064
60 day	1.77 ± 0.33	1.38 ± 0.21	1.67 ± 0.43	1.59 ± 0.42	T2	0.343	0.307	0.415

*p-value < 0.05. [1]Averaged value of negative feelings from 1 (none of the time) to 5 (all of the time). [2]Averaged value of experiencing negative health characteristics from 1 (None) to 5 (severe). [3]T1 = 30 day − pre; T2 = 60 day − pre. [4]Interaction is smoking status x treatment at each timepoint; Treatment is outcomes of Solarplast® vs. placebo across time; Smoking Status is outcomes of smoker vs non-smoker across time.

Table 3. Dietary intake of participants pre-treatment, 30 day and 60 day.

Outcome Measurement	Placebo	Solarplast®	p-value
Calories (kcal)			
Pre	1741.29 ± 419.06	1624.78 ± 406.96	0.902
30 day	1839.19 ± 513.50	1693.87 ± 380.88	
60 day	1781.71 ± 572.68	1681.06 ± 488.11	
Protein (g)			
Pre	76.77 ± 27.41	74.80 ± 22.26	0.643
30 day	77.19 ± 30.05	77.85 ± 30.41	
60 day	75.57 ± 29.43	79.61 ± 34.38	
Carbohydrates (g)			
Pre	210.19 ± 71.41	164.03 ± 45.41	0.571
30 day	230.69 ± 78.37	172.18 ± 46.56	
60 day	215.04 ± 88.96	168.03 ± 61.86	
Total Fiber (g)			
Pre	17.46 ± 7.43	14.39 ± 5.99	0.965
30 day	18.13 ± 10.28	15.36 ± 6.12	
60 day	16.99 ± 12.17	14.55 ± 5.63	
Sugar (g)			
Pre	69.49 ± 43.54	51.80 ± 21.70	0.932
30 day	74.59 ± 52.70	54.74 ± 21.13	
60 day	69.16 ± 49.42	51.33 ± 24.28	
Fat (g)			
Pre	64.65 ± 21.10	68.84 ± 23.51	0.906
30 day	67.65 ± 26.47	72.33 ± 26.04	
60 day	68.89 ± 29.45	71.38 ± 21.65	
Cholesterol (mg)			
Pre	263.46 ± 151.03	269.59 ± 111.75	0.562
30 day	239.84 ± 128.87	283.54 ± 166.42	
60 day	256.62 ± 129.03	272.73 ± 147.10	
Vitamin C (mg)			
Pre	50.02 ± 40.94	40.74 ± 17.24	0.270
30 day	54.87 ± 48.23	34.99 ± 27.40	
60 day	43.45 ± 37.88	40.26 ± 27.44	
Vitamin B_6 (mg)			
Pre	1.22 ± 0.75	1.11 ± 0.39	0.335
30 day	1.48 ± 1.14	1.16 ± 0.62	
60 day	1.59 ± 1.55	1.06 ± 0.64	
Iron (mg)			
Pre	11.38 ± 4.40	10.32 ± 3.97	0.814
30 day	11.84 ± 5.33	12.07 ± 6.06	
60 day	11.57 ± 6.00	12.50 ± 6.62	

Continued

Calcium (mg)			
Pre	671.74 ± 262.29	612.52 ± 272.83	0.079
30 day	577.98 ± 279.60	656.73 ± 276.04	
60 day	642.09 ± 297.31	616.99 ± 302.74	
Magnesium (mg)			
Pre	142.10 ± 76.75	114.10 ± 48.13	0.552
30 day	153.27 ± 81.25	134.85 ± 50.18	
60 day	150.41 ± 90.01	113.79 ± 51.27	

3.2. Safety and Tolerability

Solarplast® was well-tolerated, with no reported complaints received from subjects. No adverse events were noted, suggesting high tolerability.

3.3. Blood Pressure and Heart Rate Results

There were no statistically significant interactions between treatment groups and smoking status for the change scores for systolic blood pressure (SBP), diastolic blood pressure (DBP), or heart rate. While there were statistically significant main effects of treatment for SBP change scores (p = 0.022) and DBP change scores (p = 0.013) at timepoint 2, the differences are within normal variable values and not clinically relevant (Table 4).

Table 4. Blood pressure, heart rate, and WBC measures of participants pre-treatment, 30 day and 60 day and participants' significance change outcomes.

Measures	Placebo Mean ± SD		Solarplast® Mean ± SD		Outcomes Across Time p-value			
	Smoker n = 7	Non-Smoker n = 17	Smoker n = 9	Non-Smoker n = 17		Interaction	Treatment	Smoking Status[2]
SBP (mmHg)								
Pre	119.14 ± 14.98	115.59 ± 11.76	109.67 ± 10.68	116.94 ± 13.01				
30 day	114.86 ± 5.64	119.06 ± 11.25	113.00 ± 14.12	119.65 ± 14.73	T1[1]	0.194	0.287	0.269
60 day	114.00 ± 12.56	115.12 ± 10.36	113.33 ± 14.66	118.47 ± 13.13	T2	0.142	0.022*	0.581
DBP (mmHg)								
Pre	80.29 ± 8.75	76.12 ± 8.09	74.78 ± 10.98	77.12 ± 9.99				
30 day	75.29 ± 4.72	79.47 ± 7.51	76.67 ± 9.87	78.53 ± 10.27	T1	0.076	0.315	0.112
60 day	72.71± 10.28	74.76 ± 8.51	75.11 ± 10.78	78.94 ± 9.42	T2	0.277	0.013*	0.079
Heart Rate (beats/min)								
Pre	72.29 ± 10.80	71.24 ± 6.69	74.78 ± 12.55	70.65 ± 11.50				
30 day	76.14 ± 11.29	71.82 ± 7.66	77.00 ± 10.52	72.35 ± 12.16	T1	0.513	0.902	0.369
60 day	75.86 ± 10.93	71.12 ± 9.06	81.56 ± 10.00	72.29 ± 10.11	T2	0.763	0.301	0.070

Continued

WBC (10^6 cells/L)								
Pre	6.78 ± 2.53	4.92 ± 1.44	6.41 ± 2.05	6.60 ± 1.65				
30 day	6.74 ± 1.31	5.16 ± 1.75	6.68 ± 2.29	6.03 ± 1.24	T1	0.208	0.567	0.541
60 day	7.07 ± 1.00	5.01 ± 1.72	6.13 ± 1.37	5.97 ± 1.53	T2	0.912	0.199	0.609
LYM (10^6 cells/L)								
Pre	2.51 ± 0.54	1.88 ± 0.54	2.40 ± 0.90	2.38 ± 0.95				
30 day	2.54 ± 0.59	2.07 ± 0.73	2.39 ± 0.87	2.07 ± 0.80	T1	0.350	0.277	0.774
60 day	2.87 ± 0.71	1.96 ± 0.67	2.23 ± 0.92	2.04 ± 0.69	T2	0.785	0.057	0.370
MON (10^6 cells/L)								
Pre	0.12 ± 0.16	0.08 ± 0.04	0.06 ± 0.04	0.11 ± 0.11				
30 day	0.08 ± 0.03	0.05 ± 0.02	0.07 ± 0.03	0.18 ± 0.46	T1	0.801	0.441	0.657
60 day	0.06 ± 0.01	0.06 ± 0.04	0.06 ± 0.02	0.07 ± 0.03	T2	0.131	0.654	0.900
NEU (10^6 cells/L)								
Pre	4.15 ± 1.96	2.96 ± 1.22	3.94 ± 1.41	4.11 ± 1.11				
30 day	4.12 ± 1.05	3.05 ± 1.35	4.22 ± 2.41	3.79 ± 0.95	T1	0.314	0.878	0.494
60 day	4.13 ± 0.91	2.99 ± 1.25	3.85 ± 1.47	3.81 ± 1.26	T2	0.761	0.584	0.865
LY%								
Pre	38.83 ± 8.23	39.29 ± 9.75	38.32 ± 10.22	35.67 ± 8.74				
30 day	38.04 ± 6.28	41.04 ± 9.74	37.96 ± 14.02	34.70 ± 11.01	T1	0.533	0.648	0.700
60 day	40.77 ± 8.78	39.50 ± 8.73	37.34 ± 16.25	34.40 ± 8.48	T2	0.777	0.359	0.665

*p-value < 0.05. [1]T1 = 30 day − pre; T2 = 60 day − pre. [2]Interaction is smoking status × treatment at each timepoint; Treatment is outcomes of Solarplast® vs. placebo across time; Smoking Status is outcomes of smoker vs non-smoker across time (SBP: systolic blood pressure, DBP: diastolic blood pressure, WBC: white blood cells, LYM: lymphocytes, MON: monocytes, NEU: neutrophils, LY% % lymphocytes, MO%: percentage monocytes, NEU%: percentage neutrophils).

3.4. White Blood Cell (WBC) Distribution

No statistically significant interactions between treatment groups and smoking status at both timepoints were found for WBC, LYM, MON, NEU, LY%, MO%, or NEU%, nor were there any statistically significant main effects for treatment (Table 4).

3.5. Oxidative Stress and Inflammatory Response

Neither smoking nor daily Solarplast® administration significantly influenced Advanced Oxidative Protein Products (AOPP) or malondialdehyde (MDA), however AOPP and MDA were reduced by 13% and 15%, respectively, in smokers receiving Solarplast®, but these did not reach significance ($p > 0.05$) as determined by two-way ANOVA (Table 5).

Table 5. Plasma cytokine measures of participants pre-treatment, 30 day and 60 day and participants' significance of change outcomes.

Measures	Placebo Mean ± SD		Solarplast® Mean ± SD			Outcomes Across Time p-value		
	Smoker n = 7	Non-Smoker n = 17	Smoker n = 9	Non-Smoker n = 17		Interaction	Treatment	Smoking Status[2]
IL-1β (pg/mL)								
Pre	1.44 ± 1.24	3.85 ± 6.41	6.31 ± 11.11	6.47 ± 12.96				
30 day	1.47 ± 1.58	3.03 ± 1.86	7.30 ± 11.26	3.81 ± 6.86	T1[1]	0.476	0.820	0.254
60 day	1.45 ± 1.72	3.72 ± 5.04	10.51 ± 21.09	11.31 ± 30.70	T2	0.916	0.221	0.945
IL-6 (pg/mL)								
Pre	1.22 ± 0.87	4.09 ± 7.43	3.55 ± 2.96	4.39 ± 7.68				
30 day	1.40 ± 2.31	2.18 ± 2.42	4.94 ± 4.18	2.44 ± 4.23	T1	0.725	0.745	0.132
60 day	0.68± 0.35	1.96 ± 2.05	6.57 ± 7.87	4.05 ± 9.50	T2	0.654	0.181	0.213
IL-10 (pg/mL)								
Pre	4.87 ± 3.33	5.30 ± 6.46	4.93 ± 4.94	5.56 ± 5.16				
30 day	7.09 ± 8.20	5.78 ± 5.09	4.70 ± 3.78	5.17 ± 4.22	T1	0.562	0.222	0.485
60 day	4.40 ± 1.58	5.48 ± 5.53	6.14 ± 6.81	6.17 ± 7.69	T2	0.532	0.299	0.975
TNFα (pg/mL)								
Pre	16.98 ± 8.99	18.41 ± 22.78	20.96 ± 38.04	21.43 ± 28.07				
30 day	14.34 ± 9.12	16.46 ± 10.51	24.79 ± 50.29	16.98 ± 21.71	T1	0.259	0.613	0.338
60 day	13.11 ± 8.92	18.11 ± 20.36	30.20 ± 64.97	18.98 ± 24.22	T2	0.058	0.168	0.306
AOPP (uM chloramine)								
Pre	36.49 ± 15.49	25.14 ± 9.74	34.65 ± 13.39	23.90 ± 9.37				
30 day	38.49 ± 12.53	24.05 ± 11.01	29.28 ± 11.24	23.41 ± 6.92	T1	0.252	0.329	0.796
60 day	32.30 ± 12.22	22.37 ± 8.42	30.69 ± 13.11	22.63 ± 11.09	T2	0.859	0.809	0.567
MDA (µmol/L)								
Pre	1.72 ± 0.42	1.09 ± 0.53	1.33 ± 0.55	1.24 ± 0.38				
30 day	1.72 ± 0.47	1.17 ± 0.58	1.08 ± 0.43	1.16 ± 0.44	T1	0.722	0.120	0.364
60 day	1.57 ± 0.24	1.11 ± 0.52	1.17 ± 0.48	1.26 ± 0.59	T2	0.958	0.947	0.182
Norm IL-1β								
Pre	1475 ± 1673	1175 ± 1784	1986 ± 1807	2902 ± 5764				
30 day	8177 ± 13804	2999 ± 6006	1373 ± 1225	1628 ± 2321	T1	0.263	0.008*	0.149
60 day	7313 ± 14362	3358 ± 7339	1497 ± 1704	1539 ± 2223	T2	0.502	0.026*	0.282
Norm IL-6								
Pre	5858 ± 11468	1914 ± 3079	7157 ± 8590	4013 ± 5295				
30 day	9933 ± 10102	3211 ± 6531	3734 ± 4550	2665 ± 3086	T1	0.246	0.020*	0.874
60 day	17169 ± 34497	3062 ± 4877	7106 ± 10661	3798 ± 6478	T2	0.169	0.073	0.156

Continued

Norm IL-10								
Pre	1450 ± 3082	195 ± 406	985 ± 1222	208 ± 417				
30 day	348 ± 746	263 ± 374	968 ± 988	242 ± 644	T1	0.169	0.189	0.129
60 day	365 ± 360	572 ± 1218	939 ± 1207	170 ± 246	T2	0.099	0.437	0.138
Norm TNF-α								
Pre	529 ± 503.52	249 ± 422	621 ± 650	370 ± 394				
30 day	632 ± 726.13	105 ± 140	304 ± 358	167 ± 246	T1	0.169	0.166	0.802
60 day	352 ± 378.82	201 ± 260	217 ± 243	291 ± 307	T2	0.513	0.361	0.116

*p-value < 0.05. [1]T1 = 30 day − pre; T2 = 60 day − pre. [2]Interaction is smoking status x treatment at each timepoint; Treatment is outcomes of Solarplast® vs. placebo across time; Smoking Status is outcomes of smoker vs non-smoker across time (IL-1β, 6, 10: Interleukins 1β, 6, 10, TNFα: Tumor Necrosis Factor α, AOPP: Advanced Oxidation Protein Product, MDA: malondialdehyde).

3.6. Skin Health Results

There was a significant finding observed for only one of the measured variables assessed by the Pear 3D Plus system ($p < 0.05$) in Table 6, specific to treatment. For all endpoints, there was no significant interaction between intervention group and smoking status. There was a significant improvement in moisture at 60 days in the Solarplast group compared to placebo, regardless of smoking status ($F(1, 46) = 4.56$, $p = 0.038$, partial $\eta^2 = 0.090$). Despite the significant finding for moisture levels, no other skin variables were impacted significantly and more importantly, overall skin age did not experience a significant change.

Subjective skin health (Table 7) was also assessed using a 5-point Likert scale with 1 indicating "very bad" and 5 indicating "very good" for various skin characteristics (acne, skin appearance, blemishes, brightness, dryness, elasticity, firmness, itchiness, rash, redness, and wrinkles, and overall skin health). There were no statistically significant differences in any of the subjective skin health endpoints, with the exception of "brightness." There was a significant reduction in perceived brightness at day 60 in the Solarplast group compared to placebo, regardless of smoking status ($F(1, 45) = 5.43$, $p = 0.024$, partial $\eta^2 = 0.108$).

Table 6. Skin imaging outcomes of participants pre-treatment, 30 day and 60 day and participants' significance of change outcomes.

Measures	Placebo Mean ± SD		Solarplast® Mean ± SD			Outcomes Across Time p-value		
	Smoker n = 7	Non-Smoker n = 17	Smoker n = 9	Non-Smoker n = 17		Interaction	Treatment	Smoking Status[2]
Pore								
Pre	39.43 ± 8.30	43.13 ± 11.45	48.12 ± 9.29	48.33 ± 9.99				
30 day	39.57 ± 8.26	42.75 ± 10.90	48.47 ± 8.97	48.56 ± 10.02	T1[1]	0.514	0.276	0.177
60 day	39.57 ± 7.81	42.25 ± 11.26	48.35 ± 9.21	48.61 ± 10.15	T2	0.148	0.182	0.090
Wrinkle								
Pre	37.57 ± 8.46	39.88 ± 11.54	45.65 ± 10.26	46.00 ± 10.29				
30 day	37.57 ± 8.56	39.13 ± 11.28	46.12 ± 10.08	46.33 ± 10.26	T1	0.373	0.199	0.027*
60 day	37.71± 8.36	39.38 ± 11.24	46.00 ± 10.48	46.78 ± 10.12	T2	0.050	0.683	0.007*

Continued

Elasticity								
Pre	38.43 ± 8.68	41.13 ± 11.99	46.41 ± 9.23	47.39 ± 10.56				
30 day	38.57 ± 8.73	41.50 ± 11.53	46.41 ± 8.89	47.39 ± 10.32	T1	0.741	0.741	0.462
60 day	38.14 ± 8.24	40.75 ± 11.70	46.53 ± 9.28	47.39 ± 10.05	T2	0.965	0.747	0.229
Spot								
Pre	38.86 ± 10.32	41.37 ± 13.62	49.82 ± 9.96	49.72 ± 9.80				
30 day	38.43 ± 10.55	41.63 ± 12.76	50.53 ± 9.89	50.00 ± 9.59	T1	0.137	0.734	0.119
60 day	38.43 ± 9.68	40.75 ± 13.78	50.71 ± 9.98	50.28 ± 9.45	T2	0.878	0.539	0.005*
UV Spot								
Pre	35.29 ± 13.56	43.75 ± 13.32	51.47 ± 11.16	51.44 ± 10.79				
30 day	35.57 ± 14.18	43.75 ± 13.56	51.35 ± 11.03	51.67 ± 11.05	T1	0.541	0.958	0.859
60 day	35.14 ± 12.76	41.88 ± 15.45	51.41 ± 10.92	52.22 ± 10.45	T2	0.190	0.645	0.163
Skin tone								
Pre	39.29 ± 8.34	41.75 ± 11.74	47.35 ± 9.53	47.89 ± 9.66				
30 day	39.14 ± 8.49	42.13 ± 10.78	48.00 ± 9.54	48.44 ± 9.73	T1	0.364	0.524	0.151
60 day	39.29 ± 8.38	42.38 ± 11.56	47.76 ± 9.82	48.67 ± 9.57	T2	0.674	0.112	0.364
Porphyrin ratio								
Pre	36.43 ± 9.64	40.88 ± 11.10	48.71 ± 10.02	48.50 ± 11.16				
30 day	37.71 ± 9.27	41.25 ± 11.00	48.76 ± 9.97	49.17 ± 10.65	T1	0.093	0.734	0.296
60 day	37.43 ± 8.90	40.63 ± 11.55	49.35 ± 10.75	48.44 ± 11.14	T2	0.651	0.112	0.896
Moisture								
Pre	41.57 ± 8.22	44.63 ± 11.15	48.00 ± 9.50	50.72 ± 01.64				
30 day	41.86 ± 9.05	45.37 ± 11.10	47.00 ± 10.51	50.61 ± 10.22	T1	0.805	0.432	0.215
60 day	39.86 ± 9.62	45.00 ± 11.38	47.76 ± 10.09	52.17 ± 10.28	T2	0.818	0.038*	0.156
Skin age								
Pre	38.43 ± 9.22	42.13 ± 11.61	48.24 ± 9.42	48.78 ± 10.01				
30 day	38.57 ± 9.27	42.25 ± 11.24	48.35 ± 9.67	49.17 ± 9.90	T1	0.456	0.513	0.538
60 day	38.14 ± 8.92	41.50 ± 11.88	48.47 ± 9.59	49.28 ± 9.88	T2	0.190	0.870	<0.001*

*p-value < 0.05. [1]T1 = 30 day − pre; T2 = 60 day − pre. [2]Interaction is smoking status × treatment at each timepoint; Treatment is outcomes of Solarplast® vs. placebo across time; Smoking Status is outcomes of smoker vs non-smoker across time.

Table 7. Self-perceived skin outcomes of participants pre-treatment, 30 day and 60 day and participants' significance of change outcomes.

Measures	Placebo Mean ± SD		Solarplast® Mean ± SD			Outcomes Across Time p-value		
	Smoker n = 7	Non-Smoker n = 17	Smoker n = 9	Non-Smoker n = 17		Interaction	Treatment	Smoking Status[2]
Acne								
Pre	4.17 ± 0.98	3.94 ± 1.20	4.00 ± 0.71	4.38 ± 0.72				
30 day	4.17 ± 0.75	4.35 ± 1.06	4.11 ± 0.60	4.00 ± 1.16	T1[1]	0.121	0.244	0.925
60 day	4.30 ± 1.06	4.29 ± 1.11	4.22 ± 0.67	4.06 ± 1.00	T2	0.156	0.484	0.788

Continued

Skin Appearance								
Pre	3.67 ± 1.03	3.76 ± 0.83	3.44 ± 0.53	4.00 ± 0.63				
30 day	3.83 ± 0.98	4.00 ± 0.79	3.56 ± 0.73	3.56 ± 1.03	T1	0.351	0.269	0.473
60 day	4.17 ± 0.98	4.12 ± 0.86	3.89 ± 0.60	3.69 ± 0.95	T2	0.207	0.442	0.292
Blemishes								
Pre	4.00 ± 1.10	3.69 ± 1.25	4.11 ± 0.60	3.94 ± 0.77				
30 day	4.00 ± 0.89	3.88 ± 1.03	4.00 ± 0.71	3.75 ± 1.13	T1	0.724	0.508	0.864
60 day	4.17 ± 0.75	3.88 ± 1.26	4.11 ± 0.78	3.88 ± 1.20	T2	0.793	0.333	0.666
Brightness								
Pre	3.50 ± 1.00	3.35 ± 0.86	3.75 ± 0.71	3.53 ± 0.74				
30 day	4.00 ± 0.82	3.82 ± 0.81	3.50 ± 0.54	3.53 ± 0.74	T1	0.973	0.078	0.957
60 day	3.75 ± 0.96	4.00 ± 0.79	3.38 ± 0.74	3.47 ± 0.92	T2	0.874	0.024*	0.394
Dryness								
Pre	3.33 ± 1.51	3.00 ± 1.28	3.00 ± 1.00	3.00 ± 0.52				
30 day	3.83 ± 0.98	3.82 ± 0.81	3.44 ± 0.73	3.31 ± 0.95	T1	0.633	0.531	0.696
60 day	3.67 ± 0.82	3.71 ± 0.99	3.33 ± 0.87	3.50 ± 1.10	T2	0.809	0.918	0.325
Elasticity								
Pre	3.83 ± 1.17	3.29 ± 0.99	3.22 ± 1.09	3.19 ± 0.66				
30 day	4.00 ± 0.89	3.65 ± 0.70	3.33 ± 0.71	3.56 ± 0.81	T1	0.847	0.996	0.414
60 day	4.00 ± 0.89	3.82 ± 0.73	3.22 ± 0.83	3.50 ± 0.73	T2	0.752	0.752	0.119
Firmness								
Pre	3.83 ± 0.98	3.24 ± 1.09	3.22 ± 1.20	3.25 ± 0.68				
30 day	4.17 ± 0.75	3.65 ± 0.61	3.22 ± 0.83	3.56 ± 0.81	T1	0.685	0.562	0.524
60 day	3.83 ± 1.17	3.82 ± 0.73	3.22 ± 0.83	3.50 ± 0.73	T2	0.399	0.720	0.104
Itchiness								
Pre	4.40 ± 1.34	4.00 ± 1.12	3.44 ± 1.59	3.81 ± 1.05				
30 day	4.40 ± 0.89	4.35 ± 0.93	4.11 ± 0.78	4.19 ± 1.17	T1	0.423	0.423	0.962
60 day	4.00 ± 1.00	4.06 ± 0.97	4.00 ± 1.00	4.19 ± 0.91	T2	0.292	0.066	0.622
Rash								
Pre	4.40 ± 1.34	4.24 ± 1.09	3.56 ± 1.42	4.56 ± 0.73				
30 day	4.60 ± 0.55	4.65 ± 0.70	4.22 ± 0.83	4.31 ± 1.14	T1	0.121	0.840	0.371
60 day	4.40 ± 0.89	4.41 ± 0.80	4.22 ± 0.67	4.50 ± 0.73	T2	0.214	0.550	0.447
Redness								
Pre	4.17 ± 0.98	3.53 ± 1.18	3.67 ± 1.32	3.87 ± 1.03				
30 day	4.17 ± 0.75	4.29 ± 0.92	3.89 ± 0.78	4.00 ± 1.16	T1	0.300	0.611	0.431
60 day	4.00 ± 0.89	4.00 ± 0.89	4.33 ± 0.71	3.94 ± 1.24	T2	0.080	0.778	0.817
Wrinkles								
Pre	3.50 ± 1.52	3.53 ± 0.87	3.56 ± 1.01	3.50 ± 0.73				
30 day	3.83 ± 1.17	3.65 ± 0.61	3.56 ± 0.53	3.88 ± 0.81	T1	0.296	0.856	0.800
60 day	3.67 ± 1.21	3.82 ± 0.73	3.67 ± 0.71	3.56 ± 0.89	T2	0.723	0.642	0.863
Overall Skin Health								
Pre	4.00 ± 0.48	3.53 ± 0.80	3.67 ± 0.87	3.63 ± 0.72				
30 day	4.17 ± 0.75	4.06 ± 0.66	3.67 ± 0.50	3.63 ± 1.03	T1	0.549	0.253	0.549
60 day	4.00 ± 0.89	3.94 ± 0.90	3.78 ± 0.83	3.75 ± 0.86	T2	0.384	0.973	0.271

*p-value < 0.05. [1]T1 = 30 day − pre; T2 = 60 day − pre. [2]Interaction is smoking status × treatment at each timepoint; Treatment is outcomes of Solarplast® vs. placebo across time; Smoking Status is outcomes of smoker vs non-smoker across time.

3.7. Mental Health

In this study, mental wellbeing was assessed using the Warwick-Edinburgh Mental Wellbeing Scale (WEMWBS) with a score range of 14 - 70, where positive wellbeing is associated with higher scores. While no two-way interactions were observed between treatment and smoking status, there was a statistically significant main effect of treatment for WEMWBS T2 change scores (60 day − pre), $F(1, 45) = 9.89$, $p = 0.003$, partial $\eta^2 = 0.180$ indicating that WEMWBS scores were statistically lower at 60 day for the Solarplast® group as compared to placebo regardless of smoking status, when considering the change relative to each group's baseline value.

4. Discussion

Solarplast® is a non-GMO dietary supplement derived from enzyme treated spinach, with potential for antioxidative and anti-inflammatory effects. This study aimed to expand on previous findings by examining the effects of Solarplast® on immune function, skin health, and mental wellbeing over 60 days. Attenuation of oxidative stress and cytotoxicity has previously been demonstrated *in vitro* following treatment with Solarplast® [17]. A recent clinical trial with 45-day supplementation with 100 mg Solarplast® led to a decrease in TNF-*α* compared to placebo among healthy individuals and improved antioxidant defense and immunity, in a sample of smokers and non-smokers [18]. The present study sought to expand on those findings through examining immune function and skin health assessed analytically using the Pear 3D Plus at pre-treatment, 30 and 60 days of treatment, with mental wellbeing self-assessed weekly.

Solarplast® was well tolerated, and moderate effects on oxidative stress and immune function were observed. Changes in skin parameters were also reported that were within normal parameters and thus may not be clinically relevant. The lack of robust differences may be attributed to various lifestyle factors (e.g., physical activity, mental stress, sun exposure) that could influence outcomes within a free-living environment. Significant improvements were noted in smokers for increased moisture levels. However, these differences may at least in part be due to differences in starting mean ages for the smokers (40.4 ± 10.4 years) versus non-smokers (46.6 ± 9.7 years). Finally, and unexpectedly, the WEMWBS score at day 30 was somewhat negatively influenced by Solarplast®, which cannot be explained.

4.1. Blood Pressure and Heart Rate Discussion

Antioxidants are thought to ameliorate hypertension by reducing oxidative stress [22]. Dietary antioxidants such as those in fruits and vegetables have been found to reduce both SBP and DBP, however there is insufficient evidence that dietary antioxidant supplements provide a similar benefit [22] [23]. Despite previous studies suggesting antioxidants can reduce blood pressure, no favorable effects were observed in this study, likely due to the normotensive status of most subjects at baseline (**Table 1**). Any minor changes observed do not appear to have clinical relevance.

4.2. Biochemical Variables

White blood cell counts and distributions are altered with inflammation and disease [24]. Smoking is associated with elevated WBC counts and leads to alterations in both innate and adaptive immune responses [25]. In the present study we found no interactions for any measured parameter specific to white blood cells.

To evaluate the effects of Solarplast® on oxidative stress, AOPP and MDA were measured. While Solarplast® slightly lowered AOPP and MDA levels, these changes were not statistically significant.

Proinflammatory cytokines such as IL-1β and TNF-α increase due to oxidative stress [2]. Smokers experience more oxidative stress and therefore express higher levels of some inflammatory cytokines [26]. However, in this study no 3-way or 2-way interactions were statistically significant for any of the measured cytokines: IL-1β, IL-6, IL-10, and TNF-α (**Table 5**).

Lipopolysaccharide (LPS) can induce ROS and proinflammatory cytokines [13]. Antioxidants present in spinach may provide some protection against LPS actions [16]. To determine whether Solarplast® offered any protection, heparinized whole blood was cultured with LPS or no LPS for 6 hours and cytokine levels were measured in the culture supernatants. Normalized value for cytokines were determined by dividing the LPS stimulated levels by the non-stimulated levels. Values for normalized IL-1β were lower by approximately 30% at day 30 ($p = 0.008$) and day 60 ($p = 0.026$) with Solarplast® treatment as compared to placebo, when considering the change relative to each group's baseline value and was more pronounced for non-smokers. Values for normalized IL-6 were also lower at day 30 ($p = 0.02$) and day 60 ($p = 0.073$) with Solarplast® treatment as compared to placebo, when considering the change relative to each group's baseline value.

4.3. Skin Health Discussion

Lipopolysaccharide Antioxidants provide multiple benefits to skin health and appearance [27]. Smokers experience more oxidative stress particularly to areas exposed to smoke including the face [28]. Smoking causes a decrease in blood flow to skin and dryness, along with other adverse effects that can prematurely age skin [29] [30]. Smoking causes a decrease in blood flow to skin, along with other adverse effects that can prematurely age the skin [29]. Therefore, we hypothesized that the skin of smokers was likely to benefit more from antioxidant supplementation than the skin of non-smokers. The present study sought to extend these findings using both analytical and opinion-based measures among additional smokers and non-smokers.

As reviewed in **Table 7**, no significant statistical differences of interest were found for any of the self-assessed skin factors. However, a difference was detected via image analysis from the Pear 3D plus system (**Table 6**) for moisture levels. We noted no improvement in wrinkles with treatment and our baseline wrinkle data disagree with previous findings showing that smokers have more skin damage/wrinkles than non-smokers [31]. This may be attributed to the lower pooled

average age of smokers (40.4 ± 10.4 years) versus non-smokers (46.6 ± 9.7 years) in the present study. Considering all skin outcomes, it should be noted that subjects had good overall skin health at the start of the study, with values withing normal ranges, and therefore, had less room for improvement in response to the Solarplast® treatment.

4.4. Cognitive and Mental Health Discussion

Cognitive and mental health can be affected by oxidative stress, which can be reduced via antioxidant supplementation [32] [33]. Individuals exposed to high levels of oxidants and who are in a chronic state of oxidative stress (e.g., smokers) may be more susceptible to impaired mental health [14]. In this study, mental wellbeing was assessed using the Warwick-Edinburgh Mental Wellbeing Scale (WEMWBS) with a score range of 14 - 70, where positive wellbeing is associated with higher scores. Based on changes in scores from pre-treatment, Solarplast® resulted in slightly lower scores at both the first and second visit, as compared to pre, with scores statistically lower at day 60 for the Solarplast® group as compared to placebo group regardless of smoking status. The scores for both placebo and Solarplast® were within the average wellbeing range (42 - 60) as determined by research in the UK and therefore likely do not indicate any clinically valuable differences [22] [23].

5. Limitations and Future Directions

The study's sample size of 50 participants, although comparable to similar studies, was lower than desired, and with a more homogenous age-matched subject group would possibly yield less variability in select measures and lead to a greater chance to detect statistical significance. Additionally, because skin changes become more pronounced as we age, inclusion of an older demographic may have resulted in more robust changes in select variables. In the absence of a strong "oxidative stressor", the innate defense system of the body is usually able to regulate the balance of oxidative stress and thus may limit the likelihood of detecting a true effect of treatment.

In terms of assessing skin health and associated outcomes, a demographic of individuals with poor initial skin tone and health could be considered to determine if Solarplast® improved values as determined by questionnaires and the PEAR 3D plus system. Future studies should consider a larger, more homogenous sample of individuals with poor skin health to enhance statistical power and better understand the long-term effects of Solarplast® supplementation on associated outcomes. Additionally, more controlled dietary intake and physical activity measures would improve the reliability of the findings. Specifically, although subjects completed 5-day dietary records prior to each test day and we noted no significant differences across time in the measured variables, the recording of subjects' diets has been questioned for accuracy, with the likelihood of under-reporting. Maintaining more control of subjects' diets would be worth considering, but

not without its challenges.

6. Conclusion

Solarplast® is an enzyme treated spinach supplement, thought to have antioxidant potential. Following daily supplementation, we noted the product to be well-tolerated, with a reduction in ex vivo normalized cytokine response—specifically IL-1β. This finding is suggestive that Solarplast® may support the body's natural immune response, as both TNF-α [30] and IL-1β [31] are thought to be integrally involved in the inflammatory process. In addition, the objective measure of skin health indicated an improvement in moisture for the Solarplast group. As these data are preliminary, they should be interpreted with caution until longer-term studies are available to replicate these findings. Additional work may help to more fully elucidate the potential benefits of this ingredient on general health, oxidative status, immune support and other beneficial readouts.

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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 of University of Memphis (PRO-FY2021-416, approved July 26, 2021).

Informed Consent Statement

Informed consent was obtained from all subjects involved in the study.

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Data Availability Statement

Deidentified data may be requested by contacting the corresponding author.

Author Contributions

RJB and JP were responsible for the study conceptualization and design. JP and AS were responsible for subject recruitment, screening, and data collection. MS was responsible for data analysis. RJB was responsible for manuscript preparation. All authors were responsible for manuscript editing and all read and approved of the final manuscript.

Conflicts of Interest

RJB has received research funding from, and served as consultant to, a variety of

dietary supplement companies. No other authors declare any conflicts of interest. The sponsor had no role in the execution of the study or in the analysis of the study data.

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