

Rapid and selective mobilization of specific stem cell types after consumption of a polyphenol-rich extract from sea buckthorn berries (*Hippophae*) in healthy human subjects

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Purpose: The aim of this study was to evaluate the effects of a proanthocyanidin-rich extract of sea buckthorn berry (SBB-PE) on the numbers of various types of adult stem cells in the blood circulation of healthy human subjects.

Study design and methods: A randomized, double-blind, placebo-controlled, cross-over trial was conducted in 12 healthy subjects. Blood samples were taken immediately before and at 1 and 2 hours after consuming either placebo or 500 mg SBB-PE. Whole blood was used for immunophenotyping and flow cytometry to quantify the numbers of CD45dim CD34+ CD309+ and CD45dim CD34+ CD309– stem cells, CD45– CD31+ CD309+ endothelial stem cells, and CD45– CD90+ mesenchymal stem cells.

Results: Consumption of SBB-PE was associated with a rapid and highly selective mobilization of CD45dim CD34+ CD309– progenitor stem cells, CD45– CD31+ CD309+ endothelial stem cells, and CD45– CD90+ lymphocytoid mesenchymal stem cells. In contrast, only minor effects were seen for CD45dim CD34+ CD309+ pluripotent stem cells.

Conclusion: Consumption of SBB-PE resulted in selective mobilization of stem cell types involved in regenerative and reparative functions. These data may contribute to the understanding of the traditional uses of SBB for preventive health, regenerative health, and postponing the aging process.

Keywords: CD31, CD34, CD90, CD309, endothelial stem cells, mesenchymal stem cells

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Introduction

Over the past decade, there is a growing interest among the aging adult population to devote efforts into health maintenance instead of disease management.^{1,2} This trend happens coincidentally with an overall aging of the world population due to decreasing fertility rates combined with increased life expectancy,³ which is associated with a growing disease burden.⁴ Overall, health maintenance or disease prevention involves much lesser costs, and it is associated with much greater quality of life.^{5,6} This includes the consumption of high-quality nutrients, probiotics, vitamin and mineral supplements, botanical stem cell enhancers, and nutrient-dense fruits and berries such as amla, acai, maqui berry, and goji berry.

Another plant with a long-standing traditional use in health maintenance, as well as disease prevention and treatment, is sea buckthorn (SB). SB is a thorny nitrogen-fixing deciduous shrub native to the cold and arid regions of North Western Europe and Central Asia.⁷ Although its cultivation has spread as far as Canada⁸ and South America,⁹

SB grows naturally at high altitudes (2,500–4,000 m) on the Tibetan Plateau, Ladakh, Uttarakhand, Himachal Pradesh, Jammu and Kashmir, Sikkim, and Arunachal Pradesh regions.¹⁰ SB is highly resistant to extreme temperature and conditions of drought and abundant rain, as well as high levels of soil salinity and acidity.^{11,12} This is in part due to the fact that the roots bear bulbs that contain nitrogen-fixing bacteria, providing the development of an extensive root system in a wide spectrum of climatic conditions.¹³ For this reason, SB has been strategically propagated and cultivated in regions such as Central China to prevent soil erosion, providing an opportunity to harvest wild grown berries.

The small berries (sea buckthorn berry [SBB]), of 5–8 mm in diameter, are spread over the length of thorny branches covered with leaves. The berry consists of a dark brown seed (23% w/w), 2.8–4.2 mm long, wrapped in a juicy flesh (63% w/w) covered with a clear orange skin (7.8% w/w).^{14,15} The taste of SBB is unique and cannot be compared with any other berry or fruit, owing to its aroma due to volatile compounds such as ethyl dodecanoate, ethyl octanoate, decanol, ethyl decanoate, and ethyl dodecanoate.^{16,17}

Fruits, seeds, and leaves have all been shown to contain a wide variety of compounds known for promoting health maintenance.^{13,18} However, the actual chemical composition of the berries and leaves greatly depends on the climatic conditions, ripeness, and harvesting and processing methods.¹⁹ For example, berries grown at high altitude in harsh conditions of seasonal drought and extreme temperatures were documented to contain up to 10 times the amount of vitamin C found in berries grown at lower altitudes.⁹ Interestingly, SBB does not contain ascorbinase, which hydrolyses ascorbate, thus preserving vitamin C in the juice or dried berry.¹³ Overall, SBB is a good source of minerals, especially calcium, phosphorous, iron, and potassium, as well as vitamins C, B1, B2, E, A, and K.^{13,14,20–22} SBB is also a good source of carotenoids, mainly β -carotene, lycopene, lutein, and zeaxanthin;¹⁴ phytosterols, mainly ergosterol, stigmasterol, lanosterol, and amyrins;²³ polyphenols, notably proanthocyanidins;⁹ and flavonoids, mainly quercetin, kaempferol, myricetin, and isorhamnetin.²² Other bioactive compounds also identified in SBB include hippophae cerebroside, oleanolic acid, ursolic acid, 19- α -hydroxyursolic acid, dulcic acid, 5-hydroxymethyl-2-furancarboxaldehyde, cirsiumaldehyde, octacosanoic acid, palmitic acid, and 1-O-hexadecanolenin.²⁴

This blend of compounds provides SBB with obvious antioxidant properties that have been widely documented and even reported to be higher than 2,6-di-tert-butyl-p-hydroxytoluene (BHT) and tert-butyl-hydroxyanisole (BHA),

commonly used in food preservation.^{25,26} SBB extracts have been reported to prevent plasma lipid peroxidation, cytotoxicity, and even DNA damage.^{25,27}

Interestingly, the scientific name for SB, *Hippophae*, refers not to its physical characteristics but to its effect on health. It is derived from the Greek words “hippo” (horse) and “phae” (light) and essentially means “shining horse”. It is reported that after abandoning horses wounded in battle in a field of SB shrubs, Alexander the Great discovered on his return from battle a herd of healthy horses with shiny coats, a sign of health in a horse. SB was then brought to Greece where it has been used for centuries as feed for horses.¹⁸ A similar story has been reported with Genghis Khan who, the legend says, ordered his soldiers to harvest berries and leaves to heal faster from battle wounds.

The health benefits of SBB have been recognized for more than a thousand years in Tibetan, Mongolian, and Chinese medicine. In China, its medicinal properties were recorded in the Sibu Yidian from the Tang Dynasty and Jing Zhu Ben Cao from the Qing Dynasty. In Tibet, references to SBB were found in medicinal texts such as “rGyud-bzi” (*The Four Books of Pharmacopeia*) at the times of the Tang Dynasty (618–907AD).^{28,29} In Tibetan and Mongolian traditional medicine, SBB has been used to improve blood circulation and to treat a long list of ailments including lung conditions such as asthma, diabetes, stomach ulcers, cancer, wounds, metabolic disorders, and inflammation.^{9,18,30}

While some of the benefits on cardiovascular health and diabetes have been corroborated by Western science, few mechanisms of action aside from SBB’s antioxidant properties have been proposed to explain its wide range of health benefits. Table 1 lists the documented benefits of SB.^{31–60} Other plants associated with wide ranges of health benefits have been previously reported to act by triggering the release of stem cells from the bone marrow.^{61–63} By mobilizing more stem cells to enter the blood circulation, more stem cells are available to participate in the process of tissue repair in the body. Given the wide range of benefits associated with the use of SBB, in this study, we investigated the effect of the consumption of a proanthocyanidin-rich extract of SBB (SBB-PE) on stem cell mobilization.

Materials and methods

Study design

The clinical study followed a randomized, double-blinded, placebo-controlled crossover design. Twelve people were screened and enrolled after providing written informed consent, and this study was conducted in accordance with the Declaration of Helsinki and approved by the Sky Lakes

Table 1 Biological activities associated with SBBs

Applications	Health benefits	Mechanism of action	Reference
Cardiovascular	Inhibitory effect of SBB flavonoids on NF-kappa B activation in cardiomyocytes	Suggested antioxidant properties	31,32
	SB oil affects lipid metabolism in atherosclerosis	Mechanism unknown	33,34
	SBB antioxidants reduced risk factors for coronary heart disease in humans	Suggested antioxidant properties	35
	SBB reduced the oxidative stress in human plasma	Suggested antioxidant properties	25
	SBB reduced blood pressure, plasma lipids, and increased ventricular capillary density	Mechanism unknown	36
	SBB flavones prevented in vivo thrombogenesis	Inhibition of platelet aggregation	37
	SB seed flavones suppressed hypertension, hyperinsulinemia, and dyslipidemia	In part by improving insulin sensitivity and blocking angiotensin II	38
Gastric ulcer	SBB extract reduced ulcer number and size	May be by increasing glutathione level	39,40
	SB oil reduced ulcer	Mechanism unknown	41
	SB oil reduced ulcer in dogs	Mechanism unknown	42
Antitumor, anticarcinogenic	SB oil showed anticancer properties Isorhamnetin, a SBB flavone, had antiproliferative activity against lung cancer	Suggested antioxidant properties Upregulation of apoptotic genes	43–45
	Isorhamnetin suppressed colon cancer cell growth	Inhibition of PI3K-Akt-mTOR pathway	46
	SBB extract showed antitumor activity in in vivo two-stage carcinogenesis test in mice	Mechanism unknown	47
	SBB polysaccharide inhibited Lewis lung carcinoma	Through immunostimulation	48
Obesity	SB leaves reduced visceral fat in obese mice	Suggested antioxidant properties	49,50
	SB leaves reduced adipogenic and lipogenic gene expression	Upregulation of PPAR; reduction in acetyl-CoA carboxylase	51
Diabetes	SB seed extract had hypoglycemic effect in STZ-treated mice	Mechanism unknown	52
	SB pulp reduced glycemia, restored pancreatic beta cells in STZ-treated mice	Mechanism unknown	53
	SB leaf extract inhibited alpha-glucosidase	Inhibition of alpha-glucosidase	54
	SBB lowered fasting and postprandial glycemia by increasing insulin secretion	Mechanism unknown	55
	SBB extract suppressed postprandial peak insulin response and stabilized postprandial hyperglycemia	Mechanism unknown	56
	SB fruit oil alleviated type 2 diabetes	Through PI3K/Akt signaling pathway	57
Wound healing	SB flavones promoted wound healing	Mechanism unknown	58
	SB leaf extract accelerated wound healing	Upregulation of VEGF and angiogenesis	59
	SB seed oil help heal burn wounds	Enhance microcirculation and tissue regeneration	60

Abbreviations: SB, sea buckthorn; SBB, sea buckthorn berry; STZ, streptozotocin.

Medical Center Institutional Review Board (FWA 2603). The trial is registered at [ClinicalTrials.gov](https://clinicaltrials.gov/ct2/show/study?term=NCT03388073&rank=1) (ClinicalTrials.gov Identifier: NCT03388073). The study population included eight females and four males with an average age of 49.3 ± 21 years and a body mass index (BMI) between 20.0 and 34.6 kg/m^2 (Table 2), with no known chronic illness, frequent recreational drug use, impaired digestive function (including previous major gastrointestinal surgery), or known allergies to berry products. The study participants were scheduled for two clinic visits at least 1 week apart. The visits were always scheduled at the same time of the day for each person, the

same day of the week, and always during the morning hours of 7–11 am to minimize the effect of circadian fluctuations. Since there is a well-documented interference from exercise⁶⁴ and stress^{65–68} with the release vs homing of lymphocytes, the study environment was managed to minimize the physical and mental stress before and during testing. On arrival to each clinic visit, study participants completed a questionnaire to help monitor exceptional circumstances that might be affecting the stress level of that person on that day. Predetermined criteria for re-scheduling a visit included sleep deprivation and acute anxiety. After completing the questionnaire,

Table 2 Demographics of the study population

Study participants	Gender	Age (years)	BMI (kg/m ²)
P01	F	56	32.5
P02	F	62	23.6
P03	F	62	30.8
P04	F	32	23.5
P05	M	28	22.1
P06	M	59	29.8
P07	M	49	31.7
P08	F	61	24.5
P09	M	70	21.0
P10	F	56	22.0
P11	F	55	34.6
P12	F	51	23.3
Average		53.4	26.6
SD		12.3	4.9
Range		28–70	21.0–34.6

Abbreviations: BMI, body mass index; F, female; M, male.

volunteers were instructed to remain calm and inactive for 3 hours, comfortably seated in a chair. After the first hour, the baseline blood sample was drawn. Immediately after the baseline sample was drawn, an encapsulated test product was provided with water and consumed in the presence of the clinic staff. Blood samples were drawn at 1 and 2 hours after ingestion of the test product or placebo. At each blood draw, 6 mL of blood was drawn into sodium heparin vacutainer tubes for subsequent immunostaining. The heparin vials were placed on a rocker until staining, which was initiated within the hour of each blood draw.

Consumables

A proanthocyanidin-rich water extract of whole SBBs (Puredia, Irvine, CA, USA) was encapsulated at NIS Labs with 500 mg/dose. Placebo capsules were prepared using rice flour. Study participants and clinic staff were blinded to the consumables. Randomization was performed following the Latin Square design, such that half the study participants received the active test product at the first visit and placebo at the second visit, and the other half of the study participants received products in the reverse sequence.

Stem cell evaluation by flow cytometry

For each blood draw, triplicate samples of 100 µL of heparinized whole blood was stained using the following four-color immunostaining panel: CD31-FITC, CD34-PerCP, CD45-PO, and CD133-PE. For eight of the study participants, a fifth color was added: CD90-v421. Staining was performed

as recommended by Thermo Fisher Scientific (Waltham, MA, USA) for whole blood staining followed by a “no-wash” procedure involving Cal-Lyse® fixation of white blood cells and lysing of red blood cells. In brief, samples were stained in the dark at room temperature for 15 minutes followed by the addition of 100 µL of Cal-Lyse® Lysing solution and fixation for 10 minutes at room temperature. Red blood cells were then lysed by the addition of 1 mL of deionized water and further 10 minutes incubation in the dark at room temperature. Samples were stored at 4°C in the dark and acquired by flow cytometry within 24 hours using an acoustic-focusing Attune™ flow cytometer (Thermo Fisher Scientific). Files of 300,000–600,000 events were collected for each triplicate sample. Data on stem cell numbers were analyzed by the Attune software (Thermo Fisher Scientific) that provides results as cell per microliter of sample and compensated for the dilution factor that was part of the immunostaining protocol, so the results were converted to stem cell numbers per microliter of whole blood.

Statistical analyses

Average and SD for each data set were calculated using Excel (Microsoft Corporation, Redmond, WA, USA). The statistical significance of post-consumption changes from baseline to later assessments was evaluated by between-treatment analysis using within-subject analysis and the two-tailed paired *t*-test. Statistical significance was indicated by $P < 0.05$, and a high level of significance was indicated by $P < 0.01$.

Results

Study population and compliance

The demographic characteristics for the study participants are shown in Table 2. All 12 study participants completed the study participation with full compliance, including adhering to similar routines and food for 12 hours prior to arrival on both clinic days, remaining calm and unstressed during the 3-hour clinic visits, consuming test products with water as instructed, and allowing the three blood draws at each visit.

Post-consumption changes in circulating stem cells

Each blood sample was used to perform immunostaining and flow cytometry to evaluate the post-consumption changes to the numbers of three different subtypes of circulating stem cells. For eight of the study participants, post-consumption changes to a subset of mesenchymal stem cells (MSCs),

identified in the absence of CD45 and the expression of CD90 on cells within the lymphocyte gate were also evaluated.

Given interindividual variations in the time course of the mobilizing response following the consumption of SBB-PE, some individuals showed a greater response at 1 hour, whereas others showed a greater response at 2 hours. Therefore, simply averaging the responses at the respective time points may underestimate the actual response and provide greater SDs. For this reason, an additional analysis was performed using the data from the time point that showed the greatest response.

Changes in the number of stem cells expressing CD34

CD34 is a transmembrane protein that is expressed almost exclusively on certain types of stem cells. Lymphocytoid CD34+ stem cells are also showing a low level of expression of the protein tyrosine phosphatase receptor type C enzyme, CD45 (CD45dim). This is in contrast to mature hematopoietic cells that show a high level of cell surface CD45 expression^{69,70} and to other types of stem cells that are completely negative for CD45 (CD45-).⁷¹ The changes in CD45dim CD34+ stem cell numbers were analyzed to see whether the effects of consuming SBB-PE altered the levels of CD34+ stem cells in the circulation.

The CD45dim CD34+ stem cell population is further divided into two subtypes, based on whether the cells express the CD309 antigen, a transmembrane tyrosine kinase also known as vascular endothelial growth factor receptor-2 (VEGFR-2) and kinase insert domain-containing receptor (KDR; CD309). The presence of CD309 on stem cells has been associated with a more pluripotent (ie, undifferentiated) phenotype.⁷² Furthermore, the expression of CD309 on CD34+ cells in the circulation has been implicated with vascular maintenance and repair.⁷³ The absence of CD309 on CD34+ stem cells has been associated with a progenitor phenotype.⁷⁴ Consumption of SBB-PE triggered a selective mobilization of CD45dim CD34+ CD309- cells, in contrast to no changes to CD45dim CD34+ CD309+ pluripotential stem cells (Figure 1). When considering the greatest response at either 1 or 2 hours, the number of circulating CD45dim CD34+ CD309- cells and CD45dim CD34+ CD309+ cells increased by $24.2\% \pm 5.3\%$ ($P < 0.001$) and $19.5\% \pm 7.0\%$ ($P < 0.016$), respectively (Figure 4).

Changes in endothelial stem cells

The immunophenotyping also included the assessment of circulating endothelial stem cells with the phenotype of CD45- CD31+ CD309+. Endothelial stem cells have been shown to be rapidly mobilized following acute myocardial

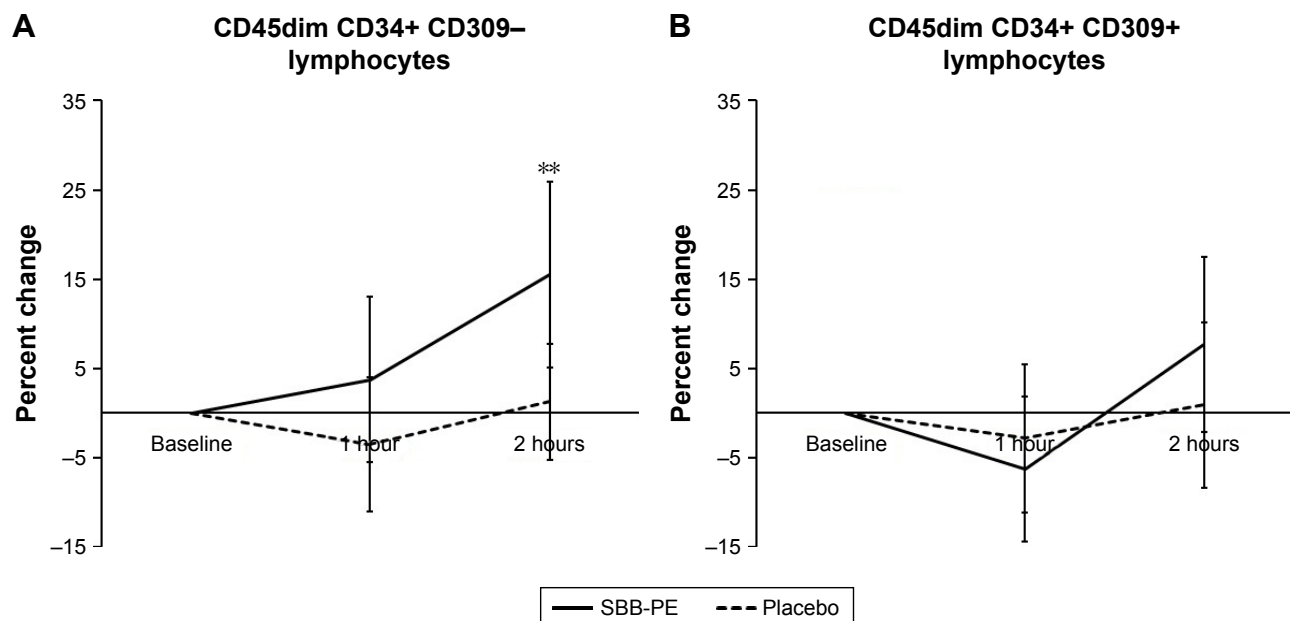


Figure 1 Changes in CD45dim CD34+ stem cells within 2 hours of consuming SBB-PE vs placebo.

Notes: The results are shown as the average $\pm$ SEM of the individual percent changes from baseline after consuming either SBB-PE (solid line) or placebo (dashed line). **(A)** For the CD45dim CD34+ CD309- lymphocytes, the difference between the treatments did not reach statistical significance ($P < 0.17$); however, the increase in CD45dim CD34+ CD309- stem cells at 2 hours after consuming SBB-PE was highly significant when comparing to baseline (** $P < 0.007$). **(B)** In contrast, there were no significant changes in the CD45dim CD34+ CD309+ stem cells after consuming either SBB-PE or placebo.

Abbreviations: SBB-PE, proanthocyanidin-rich extract of sea buckthorn berry; SEM, standard error of the mean.

infarction^{76,77} and are being actively researched for use in regenerative medicine. Consumption of SBB-PE triggered mobilization of CD45⁺ CD31⁺ CD309⁺ endothelial stem cells, in contrast to placebo where a mild reduction was seen, likely part of the normal circadian rhythm for this cell type. The difference in the numbers of circulating CD45⁺ CD31⁺ CD309⁺ endothelial stem cells after consumption of SBB-PE vs placebo was statistically significant at 1 hour ($P<0.04$) and after 2 hours ($P<0.05$; Figure 2). When considering the greatest response at either 60 or 120 minutes, the number of circulating CD45⁺ CD31⁺ CD309⁺ cells increased by $33.4\pm10.2\%$ ($P<0.007$; Figure 4).

Changes in MSCs

For eight of the twelve study participants, the changes in numbers of lymphocytoid stem cells that expressed the mesenchymal stem cell marker CD90 was evaluated. The lymphocytoid stem cells that expressed the mesenchymal stem cell marker CD90 were negative for CD45. Post-consumption changes showed an increase in CD45⁺ CD90⁺ lymphocytoid stem cells at 1 hour and 2 hours after consuming SBB, in contrast to only minor changes after consuming placebo. The increase at 2 hours, compared to baseline, reached a borderline statistical trend ($P<0.11$) (Figure 3). When considering the greatest response at either 60 or 120 minutes, the number of circulating CD45⁺ CD90⁺ lymphocytoid stem cells increased by $20.8 \pm 5.7\%$ ($P<0.016$) (Figure 4).

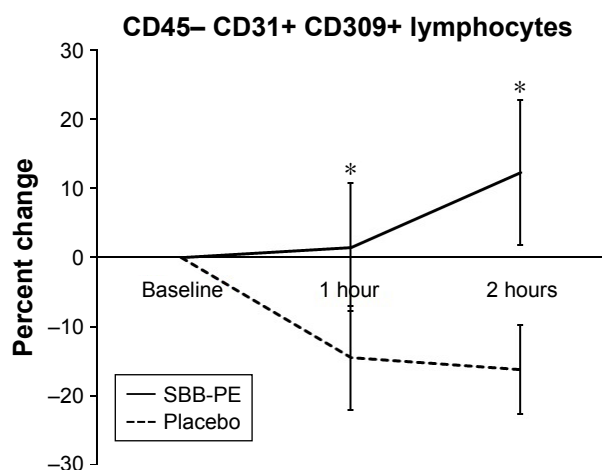


Figure 2 Changes in CD45⁺ CD31⁺ CD309⁺ endothelial stem cells within 2 hours of consuming SBB-PE vs placebo.

Notes: The results are shown as the average $\pm$ SEM of the individual percent changes from baseline after consuming either SBB-PE (solid line) or placebo (dashed line). There was no significant difference between the treatments; however, the increase in CD45⁺ CD31⁺ CD309⁺ endothelial stem cells at 1 and 2 hours after consuming SBB-PE was statistically significant when comparing to baseline ($*P<0.05$).

Abbreviations: SBB-PE, proanthocyanidin-rich extract of sea buckthorn berry; SEM, standard error of the mean.

Discussion

The migration of stem cells, seeking sites in need of repair, is crucial for the processes involved in ongoing normal maintenance and rejuvenation of healthy tissue, as well as for specific repair and healing of injured tissue. Therefore, the documentation of stem cell mobilization after consuming a botanical SBB-PE suggests a mechanism for the traditional uses of SBB in folk medicine. The basic definition of a stem cell is an undifferentiated cell that can self-replicate and also can develop into any one of various kinds of cells (such as blood cells, skin cells, nerve cells, etc.). There are multiple types of stem cells, each having distinct phenotypes. Pluripotential stem cells have the capacity to differentiate into hematopoietic and non-hematopoietic lineages. MSCs (also known as multipotent mesenchymal stromal cells) are non-hematopoietic and possess the capacity for self-renewal and multi-lineage differentiation, including osteoblasts, chondrocytes, neurons, muscle cells, adipocytes, and insulin-expressing cells in pancreatic islets. MSCs have also been demonstrated to have unique immunomodulatory properties including their ability to reduce immune cell infiltration and to modulate inflammation.

The response to consuming SBB-PE was selective and involved specific types of stem cells, whereas the numbers of other types of stem cells in the blood circulation did not change. The CD45^{dim} CD34⁺ CD309⁺ cells, CD45^{dim} CD34⁺ CD309⁺ cells, and the CD45⁺ CD31⁺ CD309⁺ endothelial stem cells showed significant mobilization above placebo within 2 hours. Regarding their regenerative properties, the number of circulating stem cells has been documented to be a critical parameter. When the number of circulating endothelial progenitor cells was quantified in the blood of 509 individuals at risk for cardiovascular disease and the incidence of cardiovascular events in these individuals was monitored for 1 year, a significantly greater number of events took place in the individuals having fewer circulating stem cells. In this study, the number of circulating stem cells was documented as one of the best predictor of cardiovascular health.⁷⁸ Likewise, following acute myocardial infarction, individuals with a higher baseline number of circulating stem cells showed a greater ejection fraction 6 months later.⁷⁹ A link between a lower number of circulating stem cells and the development of degenerative diseases has been established with diabetes,⁸⁰ cardiovascular diseases,⁸¹ atherosclerosis,^{82,83} Alzheimer's disease,^{84,85} rheumatoid arthritis,^{86,87} pulmonary diseases,^{88,89} erectile dysfunction,^{90,91} and muscular dystrophy.⁹²

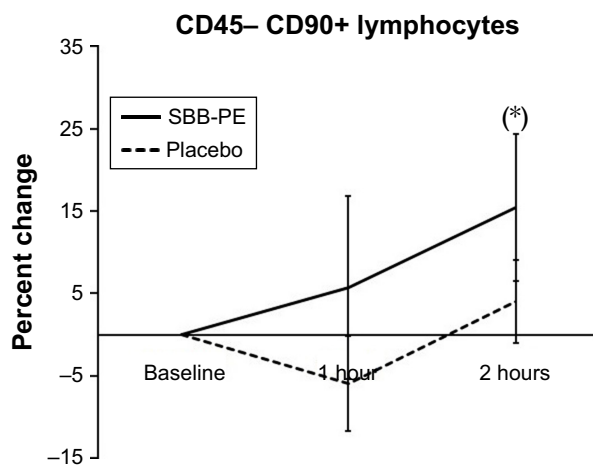


Figure 3 Changes in circulating CD90+ mesenchymal stem cell subpopulations.

Notes: The results are shown as the average $\pm$ SEM of the individual percent changes from baseline after consuming either SBB-PE (solid line) or placebo (dashed line). For the CD45- CD90+ lymphocytes, the difference between the treatments did not reach statistical significance; however, the increase in CD45dim CD90+ mesenchymal stem cells at 2 hours after consuming SBB-PE reached a statistical trend (* $P < 0.1$).

Abbreviations: SBB-PE, proanthocyanidin-rich extract of sea buckthorn berry; SEM, standard error of the mean.

Furthermore, increasing the number of circulating stem cells has been documented to enhance tissue repair or to improve the course of disease formation in cases of acute myocardial infarction,^{93,94} stroke,⁹⁵ bone fracture,⁹⁶ muscle injury,⁹⁷ spinal cord injury,⁹⁸ diabetic wound healing,⁹⁹ and inner ear damage,¹⁰⁰ to name a few. Stem cell mobilization using an extract of the cyanophyta species *Aphanizomenon flos-aquae* was documented to improve cases of severe cardiomyopathy, stroke, diabetes, rheumatoid arthritis, kidney failure, and Parkinson's disease.⁶² An extract of *Rehmannia glutinosa* was also shown to trigger stem cell

mobilization via a CXCR4-dependent mechanism and to improve the outcome of myocardial infarction.¹⁰¹ Altogether, these data suggest that SBB-PE could be a novel natural stem cell mobilizer with the potential of improving the course of several degenerative diseases.

We have shown here that consuming SBB-PE leads to selective increase in circulating CD45- CD90+ lymphocytes. Stem cells were also shown to play an important paracrine role, which includes cross-talk with other cell types and profound regulating effects such as reducing inflammation,¹⁰² which can play an important role in wound healing. MSCs expressing CD90 are capable of suppressing inflammatory conditions by various mechanisms. This has been well documented in various types of tissue models, including dental pulp stem cells that have shown immunomodulatory effects where specific inflammatory macrophage activity and cytokine profile are suppressed.¹⁰³ MSCs also support a shift in macrophage polarization from the highly inflammatory "M1" toward the anti-inflammatory "M2" type, which is known to facilitate wound healing and is also associated with changes in metabolic health and glucose metabolism.¹⁰⁴ Thus, reparative effects of stem cells in, for example, diabetes may involve repair through regeneration of pancreatic beta-cells, but may also involve an immunological shift in macrophage polarization, positively supporting an improved metabolic health.

Resolution of inflammation is essential to successful healing of wounds and damaged tissue, and chronic inflammation can lead to poor healing outcomes.¹⁰⁵ The plasticity within the stem cell hierarchy is important and even though the hierarchy places pluripotent stem cells at the top, with

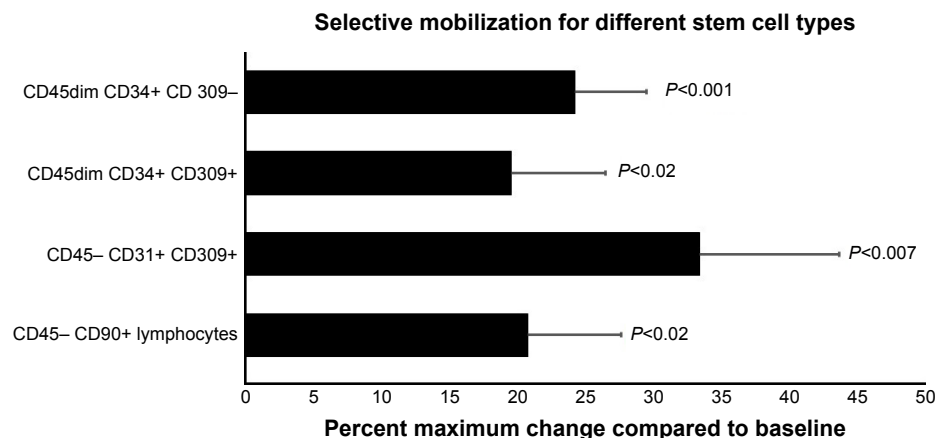


Figure 4 The maximum change (1 or 2 hours) after consumption of SBB-PE.

Notes: The maximum changes in stem cell numbers, whether seen at 1 or 2 hours after consumption of SBB-PE, were adjusted for the matching placebo response for each person, and the results are represented as average $\pm$ SEM. The analysis of the acute effects on stem cell mobilization reduced the individual variations and showed significant changes above placebo.

Abbreviations: SBB-PE, proanthocyanidin-rich extract of sea buckthorn berry; SEM, standard error of the mean.

endothelial, mesenchymal, and hematopoietic stem cells at a further differentiated state, the possibility exists to revert back to the pluripotent state.¹⁰⁶ The ability of MSCs to modulate the inflammatory response in wounds includes both a reduction in infiltration of inflammatory cells and a reduction in pro-inflammatory cytokines such as interleukin (IL)-1 and tumor necrosis factor (TNF)-alpha. This ability also supports their favorable effect on the healing response, and there is an increasing interest in non-invasive use of plant extract for regenerative medicine.¹⁰⁷ The clinical importance of stem cell mobilization in reparative functions is well documented for improvement in diabetic conditions¹⁰⁸ and cardiovascular repair after infarct,¹⁰⁹ and therefore, natural stem cell mobilizers such as SBB-PE could be effective tools to improve overall health and to accelerate the healing process by supporting the actual process of tissue repair and reducing inflammation.

Conclusion

By documenting the ability of SBB-PE to support stem cell mobilization and to increase the number of circulating stem cells, we have uncovered a new mechanism of action behind many of the health benefits that have been historically associated with SBB, as a part of its use in Tibetan, Mongolian, and Chinese traditional medicine. Once mobilized, bone marrow stem cells can participate in the process of tissue repair and modulate local inflammation, supporting the use of SBB-PE in overall health maintenance.

Data sharing statement

The authors do not plan to share individual de-identified participant data beyond the data presentation in this manuscript.

Acknowledgments

This study was conducted at NIS Labs, an independent contract research organization that specializes in natural products research. The study was co-sponsored by Biomx Stemceuticals LLC and NIS Labs R&D, Inc.

Author contributions

CD and GSJ planned the testing of sea buckthorn for stem cell effects. CD researched the historical use and current scientific knowledge about sea buckthorn. GSJ designed and oversaw the clinical study. KFB conducted the stem cell staining and flow cytometry. KFB and GSJ analyzed the data and interpreted the results. CD and GSJ co-wrote the manuscript. All authors contributed to data analysis, drafting or revising the

article, gave final approval of the version to be published, and agree to be accountable for all aspects of the work.

Disclosure

CD is the co-founder and executive director of Biomx Stemceuticals LLC, and GSJ is the research director for NIS Labs. The authors report no other conflicts of interest in this work.

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