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RESEARCH ARTICLE

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Green Tea Yogurt Supplemented with *L. paracasei* E1 Microcapsules Increases Erythrocyte Counts and B Cell Development in High-Fat Fructose Diet Mice

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Background: Obesity-induced inflammation causes hematopoietic stress, disrupting bone marrow homeostasis. Green tea yogurt supplemented with *Lacticaseibacillus paracasei* E1 microcapsules (GTYP) is a promising way to overcome obesity due to its high antioxidant and anti-inflammatory activity. However, GTYP effects on blood production, specifically erythrocytes and B cells, remain unexplored.

Materials and methods: Male Balb/C mice were fed either a high-fat fructose diet or a normal diet for 12 weeks. Microencapsulation was done by double coating of alginate-chitosan. There were seven groups in this study: normal diet (ND), high-fat fructose diet (HFFD), HFFD with 1.3 mg/kgBW Simvastatin (T1), HFFD with 5 g/kgBW plain yogurt (T2), HFFD with 2.5 g/kgBW GTYP (T3), HFFD with 5 g/kgBW GTYP (T4), and HFFD with 10 g/kgBW GTYP (T5). Erythrocyte counts from the peripheral blood were taken weekly. After 28 days of treatment, mice were sacrificed, bone marrow (BM) and lymphocytes were isolated. The cells of $Ter119^+$, $Ter119^+CD59^+$, and $B220^+SDF\text{-}1^+$ were measured using flow cytometry.

Results: HFFD not only reduces the peripheral erythrocyte count (2.15×10^9 cell/mL) but also affects the hematopoietic system, depleting $Ter119^+$ (11.76%), $TER119^+CD59^+$ (0.050%), and B220+SDF-1^+ (0.465%). Mice receiving 5 g/KgBW GTYP improved erythrocyte count (9.95×10^9 cells/mL). The parameters of erythrocyte and B cell development showed more remarkable improvement with GTYP treatment than simvastatin and plain yogurt ($p<0.05$). Molecular docking also indicated a great inhibitory effect of EGCG (-7.7) for the CXCR4 receptor.

Conclusion: GTYP can potentially increase erythrocyte count and B cell development, particularly under obese conditions.

Keywords: *anti-obesity, B lymphopoiesis, erythrocyte count, green tea yogurt, probiotics*

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Introduction

Projections for 2025 estimate that approximately 167 million people will suffer from health issues due to overweight or obesity, contributing to the chronic disease. A study of the adult populations in Indonesia revealed a high prevalence of obesity and central obesity, at 23.1% and 28%, respectively, linked to increased risk of diabetes and hypertension.[1] Excessive nutrient intake accumulates in adipose tissue, leading to remodelling of adipose tissue. This condition leads to the generation of inflammatory stimuli and promotes the infiltration of monocytes, which intensifies inflammation.[2]

Obesity has been associated with a 20-30% increase in bone marrow (BM) cellularity and altered hematopoietic lineages. Obesity-induced inflammation alters the BM population and microenvironment, affecting the quiescence of hematopoietic stem cells (HSCs).[3] Research indicates obesity appears to decrease iron serum levels compared to non-obese adolescents, which leads to the depletion of the erythrocyte count. Although iron deficiency may seem counterintuitive in obese conditions due to excessive nutrient intake, it is likely attributed to the upregulation of hepcidin levels, which impairs iron uptake.[4] Another cohort study in the pre-and post-menopausal period suggests that erythrocyte count was greater in obese individuals.[5] Therefore, obesity contributes to hematologic changes and gradually impacts individual health. CD59 is a membrane attack complex (MAC) formation inhibitor component, which is expressed by erythrocytes and other tissue. The deficiency of such protein shows susceptibility to erythrocyte hemolysis.[6]

Numerous studies have reported that long-term exposure to a high-fat diet (HFD) will suppress B-cell development by interfering with the required signals derived from stromal cells. Stromal cell-derived factor-1 (CXCL12), produced by stromal cells, was recognized as a cytokine that acts through a specific G-coupled protein receptor (GCPR), which is involved in the migration, survival, and differentiation of progenitor stem cells.[7] The interaction of CXCL12 and its receptor CXCR4 is important for B cell lymphopoiesis. Moreover, the study also reported that most of the B cell precursors and B cells at any stage of development were attached to the stromal cells, which have abundant expression of CXCL12.[8] Hence, the investigation of $B220^{+}$/$SDF\text{-}1^{+}$ was also analyzed in this present study.

Although the interaction of CXCL12/CXCR4 is important for HSC development, the overexpression of SDF-1 in the circulation enhances the mobilization of stem cell progenitors.[9] This condition presumably originated during obesity since a recent study found that the expression of SDF-1 in the spleen and thymus was considerably higher in high-fat fructose diet (HFFD) mice.[10] Higher SDF-1 levels are a potent chemoattractant for $CD34^{+}$ cells, which in obesity, HSC predisposition to develop into myeloid progenitors (NK cells, monocytes, neutrophils, and dendritic cells).[11] Thereafter, the inflammation during obesity would be exacerbated.

Yogurt-containing probiotics were suggested for suppressing inflammation-related biomarkers such as interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α), and C-reactive protein (CRP) through gut microbiota modulation in the intestine.[12] Hence, consuming yogurt may also attenuate chronic inflammation in obese individuals. Green tea yogurt has been evaluated to contain higher antioxidant activity than plain yogurt. The supplementation of green tea affects bacteria growth with two-fold higher *Lactobacillus* spp. count compared to plain yogurt.[13] Although the mechanism of green tea promoting probiotics remains unclear, the possible explanation is that green tea polyphenols influence probiotic growth. The major component of green tea, epigallocatechin gallate (EGCG), is also reported to have health benefits as anticancer, anti-inflammatory, antioxidant, and antifibrotic.[14]

The anti-inflammatory activities of green tea were reported on an HFD animal model by inhibiting inflammatory cytokine production. Moreover, the abundance of Bacteroidetes, which correlated to inflammation, also decreased after the intervention of green tea.[15] In the present study, probiotic *L. paracasei* E1 microcapsules were applied. Microencapsulation was performed to enhance the survivability rate of probiotics, which are prone to damage during manufacture and passing through the digestive tract.[16] The study about green tea yogurt supplemented with *L. paracasei* E1 microcapsules in the hematopoietic system has not been tested. Since yogurt and green tea are known for suppressing inflammatory cytokines, it would be particularly valuable to investigate their combined effect on the hematopoietic system during obesity. This study investigated the effect of green tea yogurt supplemented with *L. paracasei* E1 microcapsules (GTYP) on obesity-related hematopoietic parameters, including erythrocyte count, B cell growth factor, and inhibition of CXCR4 receptor.

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Materials and methods

Microencapsulation of L. paracasei E1

The internal layer of the microcapsules consisted of 1.5% alginate solution (Sigma-Aldrich, Missouri, USA), and the external layer was made from 0.5% chitosan in 0.1% acetic acid (Sigma-Aldrich, Missouri, USA). The *L. paracasei* E1 cultures (10^9 CFU/mL) were mixed into the alginate solution at a 1:5 ratio and homogenized for 30 minutes. The mixture was spray-dried at 130°C. The alginate beads were combined with a 1 mol/L $CaCl_2$ solution (Merck KGaA, Darmstadt, Germany) for 30 minutes to promote gelation and then centrifuged (15 minutes, 3000 rpm, 10°C). Alginate beads were combined with chitosan solution (1 gram of beads per 100 mL of chitosan) and stirred for 40 minutes to create the outer coating. Double-coated probiotic beads were collected after the mixture was spray-dried.[17]

Yogurt Preparation

Yogurt was prepared with slight modifications of the previous method.[18] Ultra-high temperature (UHT) milk (Greenfields, Malang, Indonesia) was mixed with 6% sugar (w/v) and pasteurized at 85°C for 30 minutes. The pasteurized milk was cooled until 45°C before being combined with starter cultures. Lactic acid bacteria required for producing yogurt were *S. thermophilus* (FNCC 0040) and *L. bulgaricus* (FNCC 0041) purchased from the Center for Food and Nutrition Studies, Universitas Gadjah Mada. The starter cultures were prepared in a total concentration of 3% (v/v); additionally, for yogurt making, it was also combined with 3% (v/v) of *L. paracasei* E1 acquired from the Laboratory of Microbiology, Universitas Brawijaya. The mixture was incubated overnight at 37°C. Yogurt was utilized as a starter in subsequent batch production of plain and green tea yogurt.

The green tea cultivar (*Camelia sinensis* var. sinensis) was earned from Pasir Canar Plantation in Cianjur, Jawa Barat, Indonesia. The 4% (w/v) of green tea was infused into the pasteurized milk and refrigerated overnight. The infusion was preheated at 45°C for 15 minutes and filtered before adding the starter. Next, 2% (w/v) of *L. paracasei* E1 double-coated microcapsules were added to the tea-milk mixture. Fermentation was conducted under the same conditions. Both plain and green tea yogurt used for mice treatment contains 2% *L. paracasei* E1 microcapsules.

Animal Model Design

Male Balb/C mice (*Mus musculus*) weighing ±20-25 grams, aged 5-6 weeks, acquired from the Professor Nidom Foundation, were used as an animal models. Mice were housed in laboratory conditions with a 12-hour light/dark cycle at room temperature. After a week of acclimation, mice were split into two groups: normal diet (ND) and high-fat diet (HFD). The HFD was based on modified AIN93 and HFD 32 diets, along with a 10% fructose drink.[19] The study was approved by the Research Ethics Commission of Universitas Brawijaya (155-KEP-UB-2023). Table 1 displays the composition of ND and HFD.

Mice were fed with ND and HFFD for 12 weeks before treatment. HFFD mice were confirmed obese using the Lee obesity index.[20] Mice were then divided into seven study groups (5 mice/group): normal diet (ND), high-fat fructose diet (HFFD), HFFD+1.3 mg/kgBW Simvastatin (T1), HFFD+5 g/kgBW plain yogurt (T2), HFFD+2.5 g/kgBW GTYP (T3), HFFD+5 g/kgBW GTYP (T4), and HFFD + 10 g/kgBW GTYP (T5). During the 28-day treatment, mice received their respective diets, and yogurt was prepared by dilution with sterile dH_2O. Mice were orally gavaged with 0.5 mL yogurt daily. On the last day, the Lee obesity index was re-measured. After treatment, mice were euthanized with ketamine/xylazine (KX) for tissue isolation.

Table 1. ND and HFFD feed.

Parameter	ND (100 g)	HFFD (100 g)
Moisture	14.97	7.13
Ash	10	10.56
Fat	9.12	25.11
Protein	6.23	7.14
Carbohydrate	59.68	50.05

ND = Normal diet; HFFD = High fat fructose diet.

Erythrocyte Count

Erythrocyte counts were taken before treatment and then weekly for 28 days (7, 14, 21, and 28 days) before the yogurt

was administered. Blood samples (1 µL) were obtained from the tail, mixed with PBS in a 1:1 ratio, and stored in an icebox to prevent lysis. Trypan blue (1:1) was used to stain the samples, and erythrocytes were counted using a hemocytometer under an Olympus BX53 microscope integrated with a monitor. Images were taken, and cell counts were performed using Optilab Viewer (Miconos, Yogyakarta, Indonesia).

Isolation of Bone Marrow and Flow Cytometry Analysis

Bone marrow cells were isolated from femurs and tibias by flushing the bone cavity with 1 mL PBS.[21] The flushed cells were smashed and added with PBS to a final volume of 10 mL. The solution was centrifuged (1,500 rpm, 5 min, 10°C). The pellet was then resuspended in 1 mL PBS before immunostaining. The extracellular staining used fluorescein isothiocyanate (FITC-C) conjugated rat anti-mouse CD34 (#11-0341-82, Thermo Fisher Scientific, San Diego, USA), phycoerythrin (PE)-conjugated rat anti-mouse CD59 (#17-0596-42, BioLegend, San Diego, USA), and PE-conjugated rat anti-mouse Ter119 (#116209, BioLegend, San Diego, USA). The sample was incubated with cytofix/cytoperm buffer (BD-Biosciences, Pharmingen) for intracellular staining at 4°C for 20 min. Afterwards, the sample was added with wash-perm and centrifuged at 1,500 rpm for 5 min at 4°C. The pellets were stained with intracellular antibodies, including PE-conjugated rat anti-mouse B220 (RA3-6B2, BD Biosciences Pharmingen, San Diego, USA), Peridinin-chlorophyll-protein anti-human/mouse CXCL12/SDF (#IC350C, Bio-Techne, Minneapolis, USA). The cells of $Ter119^+$, $TER119^+CD59^+$, and $B220^+SDF\text{-}1^+$ were analyzed by flow cytometry.

Datasets

The compound selection was based on LC-MS/MS data of green tea yogurt from previous research.[13,18] IT1t was selected as the control ligand for CXCR4 for its ability to inhibit the CXCL12/CXCR4 complex. The 3D structure of compounds was retrieved from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/) and converted to .pdb format using PyMOL. All ligands underwent energy minimization with Open Babel in PyRx. The CXCR4 protein (PDB ID: 4RWS) was obtained from the RCSB Protein Data Bank (https://www.rcsb.org/structure/4RWS). The protein was removed from its ligand and water molecules using Discovery Studio R2017.

Molecular Docking Interaction and Visualization

Docking was performed using Autodock Vina via PyRx 8.0.0 software. Specific docking was applied in this study, which refers to the same binding site of docking from bioactive compound and control.[22] The docking grid was centered at X: 97.4982; Y: -0.0632; Z: 37.2387, with dimensions (Å) of X: 13.3055; Y: 5.6212; Z: 8.4572. The protein-ligand interactions were visualized using PyMol and Discovery Studio R2017 software.

Data Analysis

Flow cytometry data were examined using BD Cellquest ProTM software and statistically analyzed with IBM SPSS for Windows, version 16.0, released in 2011. Shapiro-Wilk and Levene tests were used to determine the data's normality and homogeneity, respectively, with significance levels of $p>0.05$. ANOVA and Duncan tests were used at $p<0.05$ to identify significant differences between treatments.

Results

GTYP Increased Erythrocyte Count in Obese Mice

The results showed that administering GTYP for 28 days significantly enhanced the total erythrocyte count compared with the HFFD group (Figure 1). On day 28, the administration of simvastatin, plain yogurt, and GTYP in all dosages significantly increased the erythrocyte count (6.78, 7.57, 9.60, 9.95, and 7.18, respectively, $\times 10^9$ cells/mL). Meanwhile, the erythrocyte count of HFFD groups gradually decreased on the last day of the treatments (2.15×10^9 cell/mL) compared to the pre-treatments (3.15×10^9 cell/mL). The administration of 5 g/kg BW was shown to have increased erythrocytes, while using a higher dose of GTYP did not exhibit a higher erythrocyte count.

GTYP Increased the Percentage of $Ter119^+$ and $Ter119^+CD59^+$ Cells in The Bone Marrow of HFFD Mice

The administration of GTYP improved the erythrocyte count in the BM (33.43%, 27.81%, and 29.78%, respectively) compared to the HFFD group (11.76%) (Figure 2). The data indicated that GTYP was more effective than simvastatin (27.12%) and plain yogurt (19.82%). Notably, the minimum administration of GTYP (2.5 g/kgBW) had already improved the percentage of $Ter119^+$ cells.

There were significant increase in erythrocyte regulator protein in all treated groups (Figure 3). In this study, the

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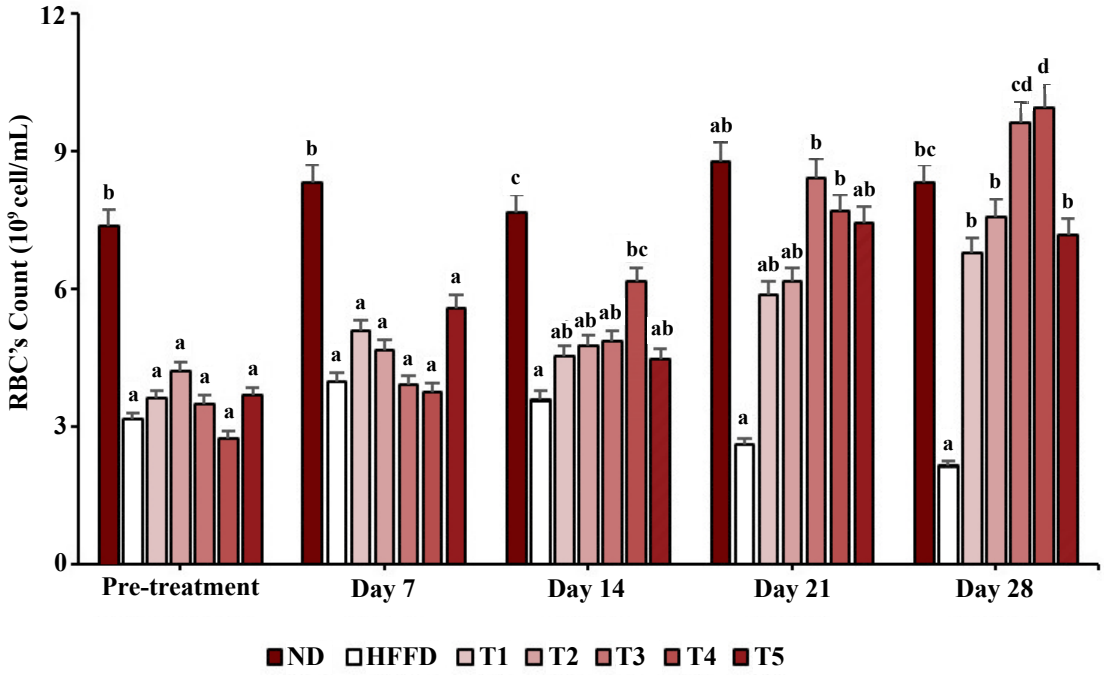

Figure 1. GTYP increased erythrocyte count in obese mice. The lowercase letters indicate significant differences across treatments (p<0.05).

HFFD treatments (0.050%) reduced the percentage of Ter119$^+$CD59$^+$ cells compared to ND (0.192%). In this case, the administration of GTYP may protect the erythrocyte membrane from damage.

GTYP Increased the Percentage of B220$^+$SDF$^+$ Cells in The Bone Marrow of HFFD Mice

B220$^+$SDF-1$^+$ was decreased after 24 weeks of HFFD feeding (0.465%) (Figure 4). All the treatments given to the HFFD mice showed an enhancement to the percentage of B220$^+$SDF-1$^+$ cells, approaching that of the ND group (0.638%). According to this findings, the administration of GTYP had a beneficial impact on B cell development in the bone marrow of HFFD mice.

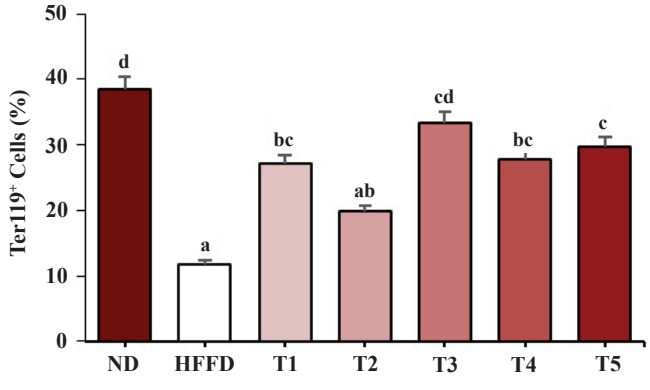

Figure 2. GTYP increased the percentage of Ter119$^+$ cells in HFFD mice's bone marrow. The lowercase letters indicate significant differences across treatments (p<0.05).

Molecular Docking Interaction of Green Tea Yogurt Compounds with CXCR4

In the present study, EGCG (-7.7) was shown to be an effective inhibitor for CXCR4 protein compared to Isothiourea-1t (-6.7) (Table 2). The other compounds found in green tea yogurt also show a promising action as an inhibitor for CXCR4. The amino acids found in green tea yogurt, including Arg188, His203, Tyr116, and Phe199, were the frequent residues to appear in every complex of ligand-CXCR4 interaction. The amino acid interaction of ligand-CXCR4 was observed in the same site (Figure 5). These data also strengthened the in vivo approach of using the GTYP for obesity.

Discussion

Obesity-induced tissue dysfunction, includinglymphoid tissues, causes an alteration in the distribution of the leukocyte subsets with greater proinflammatory phenotypes.[2] The HFFD diet caused a depletion of the erythrocyte count in the peripheral blood. The erythrocyte count recovered to normal levels after 14 days of taking GTYP. A previous cohort study on obese adults reported that the reduced erythrocytes count was due to the elevation of LDL-C, which increased free radicals and affected the erythrocyte membrane proteins.[23]

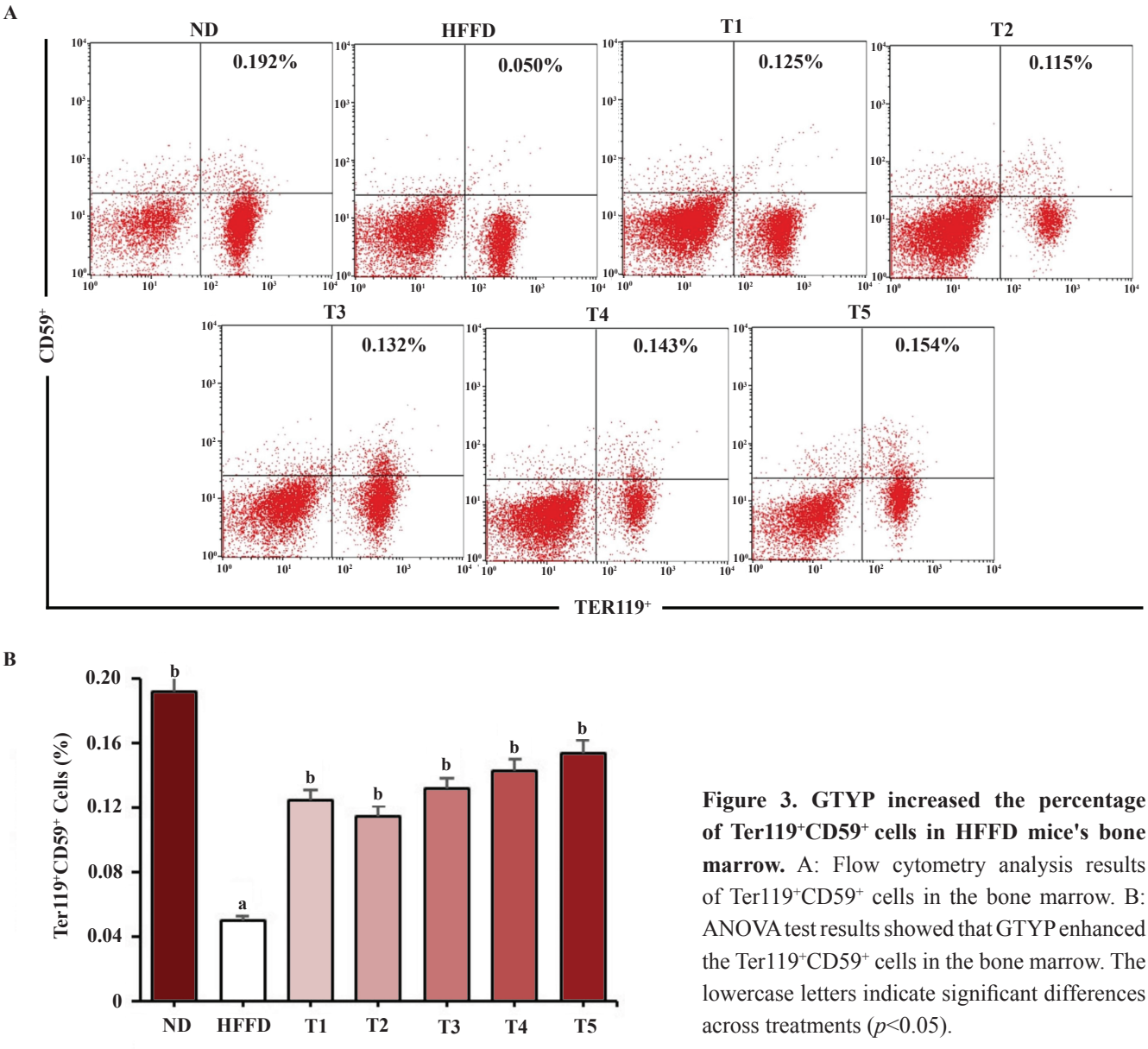

Figure 3. GTYP increased the percentage of Ter119$^+$CD59$^+$ cells in HFFD mice's bone marrow. A: Flow cytometry analysis results of Ter119$^+$CD59$^+$ cells in the bone marrow. B: ANOVA test results showed that GTYP enhanced the Ter119$^+$CD59$^+$ cells in the bone marrow. The lowercase letters indicate significant differences across treatments (p<0.05).

The study also demonstrated that obesity caused iron deficiency through dysregulating iron homeostasis through hepcidin. High expression of IL-6 during obesity leads to the elevation of hepcidin, which causes iron sequestration within the cells (enterocytes, hepatocytes, and macrophages storing iron) and reduced iron bioavailability.[4] *L. paracasei* reduces inflammation by altering the structural composition of the gut microbiota and lowering LBP levels, which is an indicator of endotoxins circulating in the blood.[24]

The examined data from the erythrocyte count in the bone marrow also indicated lower Ter119$^+$ cells in the HFFD group, which supported previous findings. This condition may occur due to hematopoietic system dysregulation that results from the interplays between obesity and inflammation. Studies have demonstrated the elevation of proinflammatory cytokines in obese rodents as a hematopoietic stressor, further promoting monocytes and disrupting the allocation of hematopoietic lineage.[25] Here, GTYP significantly restored erythrocytes to a normal level in both peripheral blood and bone marrow. Green tea is known for its rich polyphenol content, and catechin, a major compound derived from it, has the potential to act as an anti-obesity agen.[26] Compounds derived from green tea inhibited pancreatic lipase activity, which suppresses fat absorption in the small intestine.[27] Reducing body fat decreases ROS generation, leading to the downregulation of nuclear factor-

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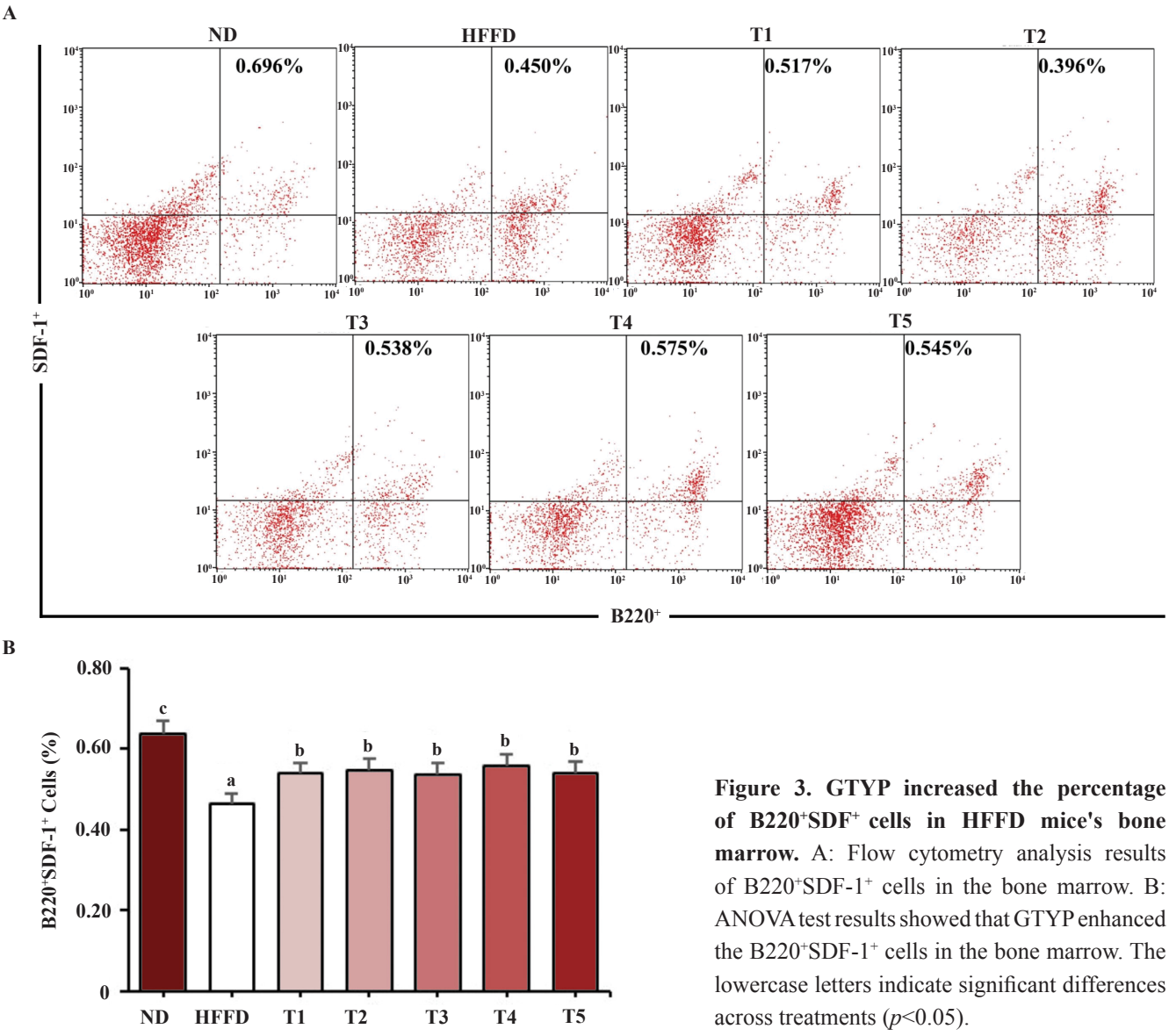

Figure 3. GTYP increased the percentage of B220$^+$SDF$^+$ cells in HFFD mice's bone marrow. A: Flow cytometry analysis results of B220$^+$SDF-1$^+$ cells in the bone marrow. B: ANOVA test results showed that GTYP enhanced the B220$^+$SDF-1$^+$ cells in the bone marrow. The lowercase letters indicate significant differences across treatments (p<0.05).

κB and a decrease in the production of proinflammatory cytokines such as IL-1β and TNF-α.[28] For this reason, the results suggested that the inflammation during obesity was alleviated, improving bone marrow homeostasis for blood cell production.

Supporting previous results that HFFD decreased erythrocyte count, the percentage of Ter119$^+$CD59$^+$ cells as a regulatory protein was also tracked. HFFD exposure further reduced the expression of the erythrocyte's regulatory protein. Recent work indicated that HFD-fed caused morphological changes in erythrocytes due to interactions with lipoproteins, resulting in lipid transfer and disruption of membrane fluidity.[29] Membrane perturbation affects various mechanisms within the membrane, including protein-lipid interactions, receptor dysregulation, and enzymatic activity. In this context, CD59, which protects against membrane damage, may be affected. CD59 blocks the membrane attack complex to prevent host cell lysis, and a lack of CD59 expression increases the vulnerability of cells to damage.[30] The fact that the level of CD59 was elevated in HFFD groups treated with GTYP implies its protective effect in maintaining the integrity of the host cell membrane. GTYP administration, from low to high doses, remained consistent with the observations in Ter119$^+$CD59$^+$. However, the peripheral erythrocyte count and Ter119$^+$ cells showed that administering the minimum dose increased both parameters.

Table 2. Interaction between green tea yogurt compounds and the CXCR4 receptor.

Ligand (CID)	Binding Affinity (kcal/mol)	Hydrogen Bond	Hydrophobic Interaction
Isothiourea-1t (25147749)	-6.7	Phe199	Arg188, Tyr116, Trp94, His113, Tyr190
Simvastatin (54454)	-6.9	Arg188, His113	His203, Leu120
Epigallocatechin gallate (EGCG) (65064)	-7.7	Arg188, His203, Val196	Arg188, His113, His203, Tyr190
Chlorogenic acid (1794427)	-6.6	His203, Phe199, Val196	
Epicatechin (72276)	-6.3	Arg188, Tyr190, Cys186	His113
Gallocatechin (65084)	-6.3	Arg188, Cys187, Glu288	His113, Tyr116, His 203
Kaempferol 3-O-glucoside (5282102)	-6.7	-	His113, Trp94, His203
Kaempferol-3-rutinoside (5318767)	-4.3	Arg188, His113, Phe199	His203, His203, Trp94

CID: Chemical identifier.

This finding suggested that even the lowest dose of GTYP may be sufficient to achieve therapeutic effects.

The investigation of the bone marrow niche also revealed that obesity affects B cell development by lowering $B220^{+}SDF\text{-}1^{+}$ cells. The interaction of SDF-1 with the CXCR4 protein receptor initiates biological processes involving the proliferation and migration of the cells.[9] However, the expression of SDF-1 was elevated due to obesity, which activated the signaling pathway of SDF-1/CXCL12-CXCR4 to induce proinflammatory macrophage production.[31] Consequently, B lymphopoiesis was suppressed due to BM-supporting capacity disruption and shift differentiation to myeloid cells (neutrophils, granulocytes, and monocytes).[32] The blockade of the SDF-1/CXCL12-CXCR4 signaling pathway improved B cell development, as shown in the present study.

The synergistic combination of yogurt and green tea generates compounds not present in green tea extract. Green tea yogurt compounds in this study exhibit potential inhibitory action compared to the CXCR4 antagonist ligand (IT1t). Interestingly, green tea yogurt compounds interacted with the same amino acid residues as IT1t (Phe199, Arg188, Tyr116, and His203). A previous study showed that Arg188-Glu277 plays a critical role in SDF-1/CXCL12-CXCR4 binding.[33] Moreover, the examined hydrogen bond between Tyr116 and Glu288 seems important for the CXCR4 activation by SDF-1.[34]

The administration of 1.3 mg/kgBW simvastatin improved regulatory protein expression and erythropoiesis during obesity. Simvastatin is known for its inhibitory activity towards HMG-CoA reductase, which reduces cholesterol production. The study also revealed potential anti-inflammatory activities of simvastatin through its inhibition of NF-κB pathway.[35] However, GTYP administration was found to be more effective than simvastatin. This may occur because simvastatin mainly acts to lower LDL concentration. Hence, it is more effective in preventing inflammation-related cardiovascular diseases such as atherosclerosis rather than directly affecting the hematopoietic system.[36]

A previous study reported that combining yogurt and 1% green tea enhances the total phenolic content, thereby increasing antioxidant activity without affecting microbial growth.[37] The health benefits of green tea yogurt were observed to prevent heart disease in men; lactic acid helps to dissolve calcium oxalate from the Krebs cycle, and green tea lowers the Krebs cycle enzyme, which reduces cardiovascular disease.[38] Moreover, *L. paracasei*, supplemented in yogurt, helped suppress the production of proinflammatory cytokines by decreasing endotoxins produced by gram-negative bacteria.[39]

These findings suggest that GTYP administration may improve the hematopoietic system in BM. The limitation of the present study is the lack of understanding of the interaction of gut microbiota with immune responses after the administration of GTYP. This speculation arises from HFD-induced changes in normal bacteria populations and yogurt might interact with the gut microbiome structure.[40] However, future research might be needed to evaluate gut microbiome structure after administering green tea yogurt supplemented with microencapsulated probiotics.

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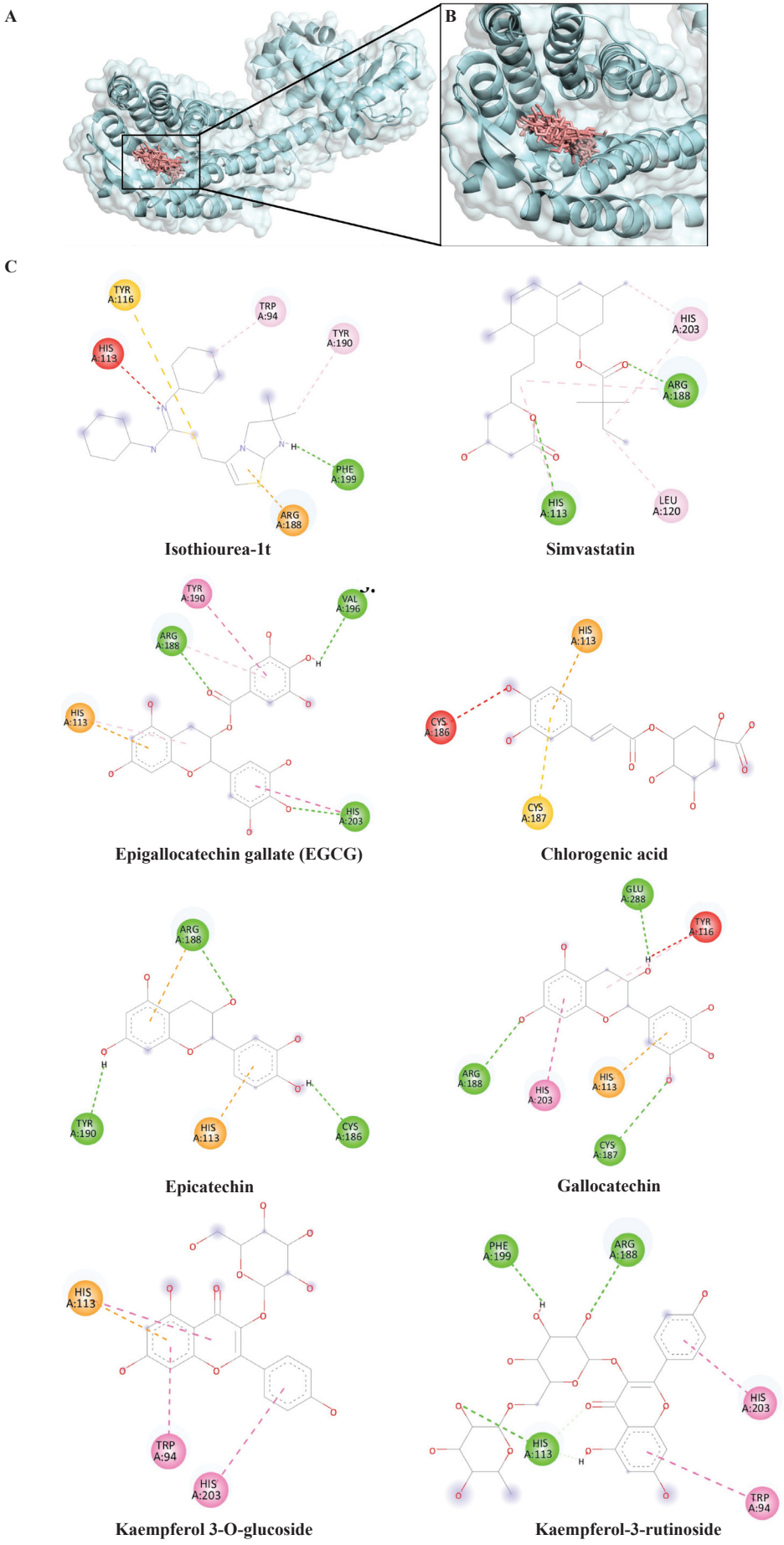

Figure 5. The interaction of ligand-CXCR4 is shown in 2D and 3D visualizations. A: Visualization of the protein surface from *in silico* docking with all ligands. B: The binding site of all ligand-CXCR4 complexes is located at the same site. C: 2D docking of interacting amino acids between ligand and CXCR4.

Conclusion

HFFD feeding significantly disrupts bone marrow homeostasis, with consequences including depletion of erythrocyte count and reduced B lymphopoiesis. Furthermore, low-dose administration of green tea yogurt supplemented with *L. paracasei* E1 (2.5 g/kgBW) has shown a protective effect towards hematopoietic outcomes by increasing erythrocyte count and promoting B cell development. The compounds in green tea yogurt act as strong inhibitors of CXCR4, especially EGCG (-7.7). From this work, it can be concluded that GTYP may help maintain the hematopoietic system in BM.

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Authors' Contributions

NAMS conducted experiments, data analysis, and wrote the manuscript. EA contributed to data analysis and wrote the manuscript. DNF was responsible for data collection and analysis. RI performed experiments and data collection. BNA was responsible for performing data collection and analysis. SAK contributed to data collection and writing the manuscript. MFA developed the methodology, collected data, and conducted data analysis. SNA contributed to conceptualizing the experiment, supervised the manuscript, and data collection. YDJ contributed to designing the study, sourcing, and supervising the manuscript. HT contributed to designing the study, sourcing, and supervising the manuscript. MR was responsible for designing the study, sourcing, and supervising the experiments. NAMS, EA, DNF, RI, BNA, SAK, MFA, SNA, YDJ, HT, and MR contributed to the critical revision of the manuscript.

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