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# Impact of Bovine Colostrum Nutraceuticals on Hematological Indices and Ratios in Elderly Patients with Type 2 Diabetes: A Single Blind Randomized Clinical Trial

**Hayder F. Lateef[1], Hayder Hdnan Fawzi[2], Talib Saddam Mohsin[3], Satmbekova Dinara[4], Datkhayev Ubaidilla[5], Zainab A Abbood[6], Ahmed Al-Basri (Co-author)[7]**

[1]Al-Zahraa Teaching Hospital, Wasit, Iraq, Hf_nice@yahoo.com

[2]College of Pharmacy, AlMustafa University, Baghdad, Iraq, Hayder.adnan2010@gmail.com https://orcid.org/0000-0001-9970-0119

[3]physiologist, Department of Anesthesia Techniques, college of health and medical, techniques, University of Kut, Wasit. IRAQ, talib.saddam@alkutcollege.edu.iq

[4]Al Farabi Kazakh National University, dskanatovna@gmail.com, https://orcid.org/0000-0003-1087-3932

[5]Asfendiyarov Kazakh National Medical University, u.datkhayev@kaznmu.kz, https://orcid.org/0000-0002-2322-220X

[6]Ministry of health, Directorate Wasit of Health, Wasit , Iraq, Zainabaa87@yahoo.com, https://orcid.org/0000-0001-7188-7743

[7]Department of Pharmacy, Al-Kut University College, 52001-Wasit, Iraq, Ahmedalbasri28@gmail.com, https://orcid.org/my-orcid?orcid=0000-0003-3592-8653

***ABSTRACT***

*A group of metabolic diseases known as diabetes mellitus is characterized by hyperglycaemia brought on by deficiencies in either insulin secretion, insulin action, or both.*

*People who are of old age frequently use several modalities of nutraceutical supplements to improve their general health. To ascertain the impact of oral bovine colostrum (BC) nutraceutical intake on the blood cell components and hematological ratios in participants undergoing their disease. A randomized, placebo-controlled trial was conducted at Al-Karama Teaching Hospital, Wasit, Iraq, the trial commenced on August 20, 2024, and was completed on December 10, 2024. 62 Elderly Patients with Type 2 Diabetes were divided into two groups: Group I, which received a placebo, and Group II, which received an oral dose of 500 mg BC capsule per day for eight weeks. At the beginning and completion of the study, the anthropometric and homological indicators were calculated. The anthropometric parameters in both groups did not significantly change. The number of granulocytes, mean corpuscular volume, mean platelet volume, and platelet large cell ratio all decreased in Group II individuals, while hemoglobin and mean corpuscular hemoglobin concentration increased. Compared to Group II, reciprocal changes in the granulocyte-to-lymphocyte and platelet-to-lymphocyte ratios were seen. We conclude that ovine colostrum is an advantageous nutritional supplement for athletes since it raises hematological ratios and indices.*

***Keywords:*** *Bovine colostrum; Elderly Patients, Type 2 Diabetes, hematological indices, hematological ratios*

## INTRODUCTION

Type 2 Diabetes (T2D) is characterized by aged, overweight, or obese individuals who exhibit insulin resistance, resulting in functional impairment of β-cells and subsequent insulin deficiency. Patients often present with comorbidities, especially cardiovascular conditions, and may initially be treated with oral antidiabetic agents; nevertheless, insulin therapy becomes essential at a later stage of disease progression(1). In insulin resistance, the secretion of the hormone from pancreatic islet cells does not effectively promote glucose absorption in metabolic tissues, leading to elevated blood glucose and insulin concentrations. Type 2 diabetes begins with a progressive decline in insulin effectiveness. In this condition, the body is unable to effectively utilize insulin for glucose transport into tissues, leading to the desensitization of insulin receptors. In reaction to insulin resistance, the body produces surplus insulin to sustain glucose homeostasis. Insulin resistance is a pathological condition marked by reduced sensitivity to insulin in target tissues. Elevated blood insulin levels in insulin resistance lead to dyslipidemia, hypertension, and changes in glucose metabolism(2). Type 2 diabetes is considered an immunological and inflammatory disorder Type 2 diabetes is considered an immunological and inflammatory disorder (3). Macrophages release cytokines, whereas chemokines provoke systemic inflammation that damages pancreatic islet cells and exacerbates insulin resistance in the liver, adipose

tissue, and skeletal muscle (4).Insulin resistance disrupts glucose and lipid metabolism, resulting in increased free fatty acid concentrations and the subsequent induction of significant amounts of reactive oxygen species (ROS) and reactive nitrogen species (RNS) (5).Free radicals generated by cellular oxidative stress are highly reactive chemical species with one or more unpaired electrons. Increased free radicals can damage cellular proteins, lipids, and nucleic acids(6). Bovine colostrum is proven to be a safe nutritional supplement for infants and children because it promotes gut function through different mechanisms, including antimicrobial, immunomodulatory, and antioxidant effects. Besides, it contains several growth factors and vitamins (7). Lactoferrin (LF) is one of the BC constituents, which is a multifunctional protein that promotes health (8). There are two possible mechanisms of LF or its derived peptides in producing its effects, which include an interaction of LF with the cell membrane receptors or its being translocated into the nucleus and modulating the target genes (9). It has been reported that taking LF supplements can prevent intestinal infections and subsequent sepsis in preterm infants without causing any side effects (10). The beneficial effects of LF are not restricted to newborns and infants but extend to adults as well. The treatments utilized to treat COVID-19 were found to be strengthened by their antiviral and anti-inflammatory properties (11). The ability of LF to bind iron is an explanatory mechanism for the effects of LF on the haematological indices (12). A recent systematic review and meta-analysis showed that LF supplements improved serum iron and ferritin, and haemoglobin, but they reduced functional iron absorption from the intestine in patients with iron deficiency anaemia (13). The beneficial effects of LF on iron deficiency anaemia are well observed in patients with inflammatory disorders, e.g., inflammatory bowel disease [9, 10]. Another study suggested a combination of LF and iron in the management of iron deficiency anaemia, which increased the storage of iron (14). In animal studies, BC was found to induce haemolytic anaemia (15), and literature surveys do not show any studies carried out on the effects of BC on the blood cells of humans. Physical activity has been proven to have a major impact on blood cells, resulting in a decrease in white blood cell count, an increase in oxygenated haemoglobin and platelet functions, and other changes (16). This study tested the hypothesis that BC, which contained natural LF, may produce variable effects on the blood elements. This study aimed to show the effects of a single oral dosage of BC nutraceuticals on the haematological indices and ratios in elderly Patients with Type 2 Diabetes.

## MATERIALS AND METHODS

### Study design

A prospective, single blind, placebo-controlled, randomized, parallel-group clinical trial was conducted, enrolling 80 patients with type 2 diabetes mellitus and carried out at Al-Karama Teaching Hospital in Wasit, Iraq, commenced on August 20, 2024, and was completed on December 10, 2024.

### Eligibility criteria

62 elderly patients with controlled type 2 diabetes on treatment and new cases (aged 65 and older), including both sexes. According to "Standards of Care in Diabetes" in the American Diabetes Association guidelines in 2023, levels of glycated hemoglobin A1c ≥6.5 are classified as diabetic mellitus (new onset) (10), or already established type 2 diabetes on regular treatment. The following were excluded from the trial: patients with complicated diabetes, chronic kidney and liver diseases, and those with terminal illnesses.

These participants were recruited from Al-Karama Teaching Hospital, Wasit, to participate in this study. For eight weeks, they typically controlled diabetes. Written consent was obtained from each participant. The researchers interviewed each participant and discussed with them the study protocol in detail. The participants are free to discontinue the study at any time. The research protocol was approved by the Ethics Committee of Al-Karama Teaching Hospital, Wasit, Iraq, (approval number: KarH240023, date of approval: 1 July 2024) according to the guidelines of Helsinki.

### Sample size

The sample size was calculated by using the G*Power 3.1 program for Windows. To demonstrate the difference between the means of paired data, the sample size was determined at a type I error (an error probability of 0.05) and a type II error (1- error or the power) of 0.95. A sample size of 22 participants for each treatment was found to be eligible for statistical analysis.

The participants were divided equally into two groups:

Group I (n=31): treated with a placebo

Group II (n=31): was treated with oral doses of BC nutraceuticals (500mg each capsule) daily for eight weeks. The BC nutraceuticals were obtained from the local pharmacies.

**Measures**

The researchers interviewed the participants, and demographic characteristics were obtained. The anthropometric measurements, including the weight (kg) and height (cm), were measured using a stadiometer, and the waist circumference (cm) was measured at the level of the umbilicus using a metallic tape measure. The following anthropometric indices were calculated:

Body mass index (BMI) = $\frac{\text{Body weight (kg)}}{\text{Squared heigh (m)}}$

Waist-to-height ratio (WHeR) = $\frac{\text{waist (cm)}}{\text{Height (cm)}}$

Lean body mass (LBM) = (0.32810×weight) + (0.33929×height)-29.5336

Where weight in kg, and height in cm. (17)

**Laboratory investigations**

Two blood samples were obtained from each participant, the first at the time of entry into the study and the second at the end of the clinical trials. The blood samples were drawn into anticoagulant test tubes for the determination of the complete blood count by using a Coulter machine. Haematological indices were included while blood cell (WBC), granulocytes, lymphocytes, other types of white blood cells not classified as lymphocytes or granulocytes (MID), red blood cell (RBC), haemoglobin (HB), haematocrit (Hct), mean cell volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), red distribution width (RDW) CV: coefficient variation, SD: standard deviation, mean platelet volume (MPV), platelet distribution width (PDW), plateletcrit (PCT), platelet large cell ratio (PLCR), and platelet large cell coefficient (P-LCC).

The granulocyte-to-lymphocyte (GLR) and blood platelet-to-lymphocyte (PLR) ratios were calculated simply by dividing the absolute number of granulocytes or platelets as a numerator and the number of lymphocytes as a denominator.

Granulocyte-to-lymphocyte ratio (GLR) = $\frac{\text{Absolute number of granulocytes}}{\text{Absolute number of lymphocytes}}$

Platelet-to-lymphocyte ratio (PLR) = $\frac{\text{Absolute number of blood platelets}}{\text{Absolute number of lymphocytes}}$

**Statistical analysis**

The results are expressed as numbers, percentages, mean, median, and standard deviation. The data were analyzed by using the Excel 10 software program for Windows (Microsoft Corporation, Redmond, Washington, USA). The defining characteristics of Groups I and II at the time of study entrance were compared using an independent two-sample t-test for continuous data and a Chi-square test for categorical data. A two-tailed paired t-test was used to compare the effects of each treatment on anthropometric and haematological indices. A p-value of 0.05 was considered to denote statistical significance.

## RESULTS

**Characteristics of the participants**

Ten out of 52 participants were drawn from the study (seven from Group I and three from Group II). Therefore, the adherence rate is 80.8%. Table 1 shows non-significant differences between Groups I and II in the age (p=0.2), residency (p=0.052), smoking (p=0.862), weight (p=0.19), height (p=0.708), BMI (p=0.558), WHeR (p=0.071), and LBM (p=0.556). The participants in Group II had a significantly higher mean value of waist circumference (p=0.024).

**Effects of BC supplement on the anthropometric measurements**

Table 1 showed that neither placebo nor BC induced significant changes in the anthropometric measurements.

**Effects of BC supplement on the white blood cells**

Both the placebo and the BC treatments produced a reduction in total WBCs. In Group I, total WBCs decreased non-significantly by 0.9% compared with 8.7% in Group II, which approximates a significant level (p = 0.055). This decline is related to the significant reduction of granulocytes in Group II compared with Group I (13.7% versus 3.1%). In Group I, there was a significant increase in the other types of

WBCs (neither granulocytes nor lymphocytes), while this effect was not observed when the participants supplemented with BC.

**Effects of BC supplement on the red blood cells**

In Group II, BC supplement significantly improves the mean hemoglobin level and the MCHC, and reduces the MCV and RDW (SD) % in elderly patients with Type 2 Diabetes. In Group I, the mean MCV value significantly decreased by 1%.

**Effects of BC supplement on the blood platelets**

Significant changes in the platelet indices were observed in the MPV, PDW%, and P-LCR in Group I, who were treated with placebo and with Type 2 Diabetes (Table 1). Participants treated with BC supplement (Group II) showed a significant reduction in the mean values of MPV and P-LCR.

**Effects of BC supplement on the granulocyte or platelet-to-lymphocyte ratios**

Figure 1A shows that the mean value of GLR is non-significantly increased by 9.8% in Group I participants compared with a non-significant reduction of 12.9% in Group II. The mean value of PLR significantly increased by 12.2% compared with a non-significant reduction of 2.7% in Group II participants (Figure 1B).

## DISCUSSION

The results of this study showed that using BC supplements by elderly patients with Type 2 Diabetes is of benefit in the hematological indices and ratios, while its effects on the anthropometric indices are non-significantly different from the placebo-treated group.

The changes in the body mass index and lean body mass may be attributed to the effect of good control of diabetes rather than to the placebo or BC supplement. Controlling diabetes intensity and duration are key determinants of changes in BMI and LBM (17). Using BC for 12 weeks significantly reduced the body weight without producing a significant effect on the LBM (18). Moreover, a specific percentage of the subjects had a considerable drop in body weight, and the effects of controlling diabetes on body weight or BMI were marginally significant. Although the improved lifestyle activates the immune system, which is characterized by increasing the number of neutrophils and monocytes and reducing the number of lymphocytes, our findings showed reciprocal changes in the granulocyte number in Group I (19). Contrary to earlier research that found utilizing BC for 4 weeks as a nutritional countermeasure supplement to prolonged control of diabetes did not have a significant impact on the number of neutrophils, BC supplements dramatically lowered the number of granulocytes (20). BC supplements significantly increase haemoglobin and decrease the RDW and MCV. Our findings do not agree with a previous study that was carried out on six elite female rowers using 60 mg/day of BC, which showed that the haemoglobin level was non-significantly increased (21). This is due to the differences in the supplemented dose of BC. The significant changes in the MCV and RDW are related to the effects of lactoferrin in the BC, which captures the heme iron and thereby reduces the diameter and size of red cells (22). Significant changes in the MPV and P-LCR (an indicator of active platelets) in both treated groups showed that controlling diabetes, not the use of a placebo or BC, was the cause. Our findings agree with many studies that diabetic controlled patients are of benefit in reducing the MPV, which is utilized as a biomarker of many clinical conditions. The reciprocal alterations in GLR and PLR following the use of BC pills highlight this study's fascinating findings. Several studies applied the determination of GLR and PLR ratios as an indication of inflammatory processes (23). As a result, taking a BC supplement encourages the immune system to control inflammation, as seen by a decrease in inflammatory bio- and haematological markers (24). One of the limitations of the study is that the effect of BC on untrained participants wasn't studied. The strength of this study is that the sample size is reasonable compared with other studies, and the participants used small dosages, which didn't produce any troublesome adverse reactions (25).

## CONCLUSIONS

We come to the conclusion that using small doses of BC supplements for elderly type 2 diabetic patients is safe and advantageous to the hemopoietic system.

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**FUNDING:** The authors declare that they have not received any funding.

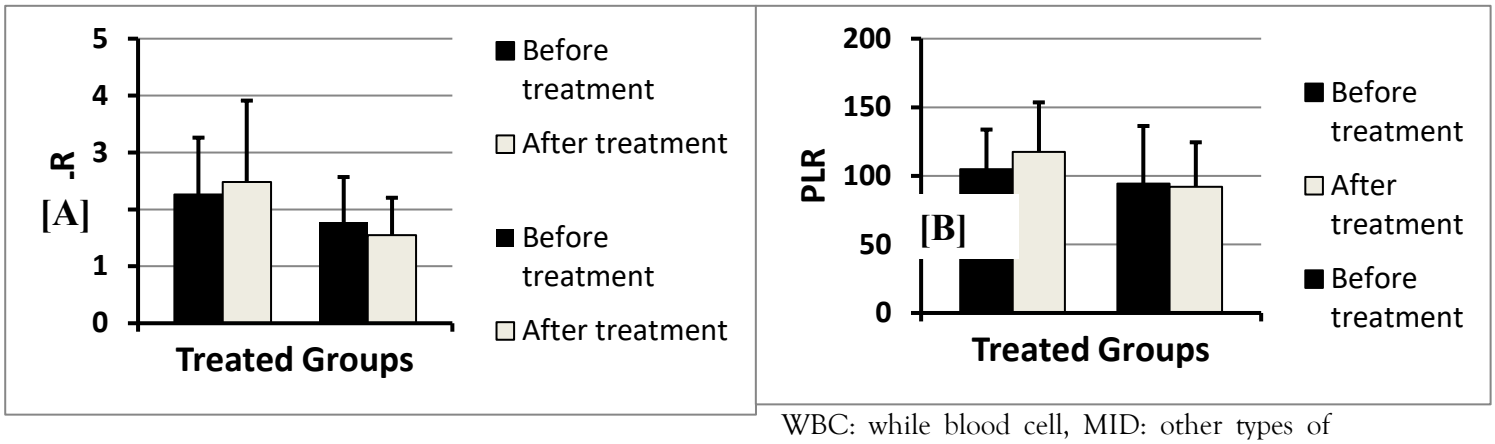
**Table 1:** Characteristics of the participants

| Variables | Placebo treatment (n=24) | | | Colostrum treatment (n=28) | | |
|---|---|---|---|---|---|---|
| | Before treatment | After treatment | p-value | Before treatment | After treatment | p-value |
| Age (year) | 75.5±5.51 | | | 75.7±9.0 | | |
| Residency | | | | | | |
| Urban | 19 | | | 27 | | |
| Rural | 5 | | | 1 | | |
| Smoking | | | | | | |
| Current | 10 | | | 11 | | |
| Non-smokers | 14 | | | 17 | | |
| Weight (kg) | 83.92±12.24 | 83.58±12.29 | 0.484 | 88.35±11.75 | 87.37±12.86 | 0.717 |
| Height (cm) | 174.33±16.08 | | | 173.11±5.59 | | |
| Waist circumference (cm) | 85.75±7.81 | 86.17±8.03 | 0.741 | 91.37±9.31 | 91.07±8.68 | 0.855 |
| Body mass index (kg/m$^2$) | 28.44±8.45 | 28.32±8.45 | 0.422 | 29.48±3.68 | 29.33±4.65 | 0.871 |
| Waist-to-height ratio | 0.497±0.069 | 0.500±0.08 | 0.655 | 0.53±0.06 | 0.528±0.057 | 0.954 |
| Lean body mass (kg) | 57.15±7.67 | 57.04±7.71 | 0.484 | 58.19±4.86 | 57.78±4.61 | 0.667 |

The results are expressed as a number and mean ± SD. P-value was calculated using a two-tailed paired t-test for continuous data.

**Table 2: Hematological indices**

| Variables | Placebo treatment (n=24) | | | Colostrum treatment (n=28) | | |
|---|---|---|---|---|---|---|
| | Before treatment | After treatment | p-value | Before treatment | After treatment | p-value |
| WBC (×10$^3$/ µL) | 7.88±1.94 | 7.81±2.36 | 0.904 | 8.61±1.86 | 7.86±1.74 | 0.055 |
| Granulocyte | | | | | | |
| No. (×10$^3$/ µL) | 5.10±1.76 | 4.94±2.12 | 0.773 | 4.96±1.45 | 4.28±1.44 | 0.038 |
| (%) | 62.33±7.59 | 60.54±10.79 | 0.458 | 55.66±11.32 | 52.19±9.23 | 0.175 |
| Lymphocyte | | | | | | |
| No. (×10$^3$/ µL) | 2.37±0.59 | 2.21±0.69 | 0.156 | 3.10±1.10 | 2.93±0.72 | 0.318 |
| (%) | 31.61±6.96 | 30.32±9.56 | 0.556 | 36.07±8.54 | 38.6±8.87 | 0.155 |
| MID | | | | | | |
| No. (×10$^3$/ µL) | 0.41±0.12 | 0.67±0.33 | 0.002 | 0.67±0.47 | 0.66±0.18 | 0.941 |
| (%) | 6.07±1.46 | 9.14±2.67 | <0.001 | 8.27±5.12 | 9.21±2.07 | 0.401 |
| RBC (×10$^6$/µL) | 5.46±0.44 | 5.43±0.44 | 0.543 | 5.31±0.41 | 5.42±0.36 | 0.115 |
| HB (mg/dL) | 14.88±0.93 | 14.73±1.15 | 0.255 | 14.52±1.37 | 14.96±1.35 | 0.017 |
| Hct (%) | 46.15±2.37 | 45.54±2.97 | 0.172 | 44.63±3.85 | 45.01±3.26 | 0.500 |
| MCV (fL) | 85.1±6.3 | 84.23±6.56 | <0.001 | 84.08±4.26 | 83.25±5.02 | 0.024 |
| MCH (pg) | 27.35±2.37 | 27.2±2.5 | 0.319 | 27.26±1.82 | 27.6±2.29 | 0.074 |
| MCHC (g/dL) | 32.18±0.77 | 32.29±1.18 | 0.597 | 32.48±0.95 | 33.16±1.12 | <0.001 |
| RDW % (CV) | 13.93±1.09 | 13.78±0.74 | 0.424 | 13.95±0.79 | 14.1±1.25 | 0.528 |
| RDW % (SD) | 47.05±3.95 | 46.98±3.57 | 0.885 | 45.43±2.86 | 47.44±2.77 | 0.001 |
| Platelets (×10$^3$/ µL) | 238.04±55.62 | 241.46±45.37 | 0.608 | 257.1±61.14 | 253.1±56.79 | 0.730 |
| MPV (fL) | 8.60±0.63 | 8.38±0.72 | 0.017 | 8.84±0.89 | 8.63±0.8 | 0.035 |
| PDW % | 11.4±0.99 | 10.95±0.84 | 0.003 | 11.79±1.41 | 11.54±1.6 | 0.237 |
| PCT (%) | 0.199±0.043 | 0.196±0.037 | 0.592 | 0.22±0.048 | 0.211±0.045 | 0.395 |
| P-LCR (%) | 22.15±4.78 | 20.43±4.98 | 0.017 | 23.71±6.1 | 21.94±6.01 | 0.017 |
| P-LCC (%) | 51.25±11.71 | 48.46±12.5 | 0.131 | 58.61±13.5 | 53.64±13.74 | 0.093 |
| GLR | 2.261±1.000 | 2.482±1.430 | 0.534 | 1.775±0.794 | 1.546±0.659 | 0.172 |
| PLR | 104.77±28.96 | 117.51±36.04 | 0.029 | 94.44±41.9 | 91.89±32.56 | 0.662 |




WBC: while blood cell, MID: other types of white blood cells not classified as lymphocytes or granulocytes, RBC: red blood cell, HB: haemoglobin, Hct: haematocrit, MCV: mean cell volume, MCH: mean corpuscular haemoglobin, MCHC: mean corpuscular haemoglobin concentration, RDW: red distribution width CV: coefficient variation, SD: standard deviation, MPV: mean platelet volume, PDW: platelet distribution width, PCT: plateletcrit, P-LCR: platelet large cell ratio, PLCC: platelet large cell coefficient, GLR: granulocyte-to-lymphocyte ratio, PLR: platelet-to-lymphocyte ratio

*

Figure 1 shows the effect of placebo treatment (Grou e left side and bovine colostrum supplement (Group II) on the right side of each figure on [A] the granuıocyte-to-lymphocyte ratio (GLR) and [B] the platelet-to-lymphocyte ratio (PLR). * p=0.029.

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