Effect of Mannan Extracted from *Saccharomyces boulardii* on Hematological and Immune Parameters in Rats with High-Fat Diet–Induced Metabolic Stress

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Abstract

A high-fat diet is a major contributor to metabolic and inflammatory disorders that negatively affect hematological and immune parameters. This study aimed to evaluate the physiological effects of Pre-extracted and purified mannan extracted from the cell wall of *Saccharomyces boulardii* in rats subjected to high-fat diet–induced metabolic stress . Thirty-two male albino rats (Rattus norvegicus) were randomly assigned into eight groups (n= 4 per group) control (G1), high-fat diet (G2), mannan-treated groups at doses of 25, 50, 75, and 100 mg/kg (G3 to G6), atorvastatin-treated group (G7), and a standard diet with mannan (G8). Hematological parameters including RBC, HGB, PCV, MCV, MCH, WBC, lymphocytes (LYM), and platelets (PLT) were assessed. The high-fat diet (G2) exhibited significant alterations, including increased WBC (10.86 $\times 10^3$ cells/mL) and PLT (395.8 $\times 10^3$ platelets/mL) alongside decreased RBC (7.793 $\times 10^6$ cells/mL) HGB (9.79 g/dL) PCV (38.52%) and MCH (12.50 pg) compared with the control (G1), which showed normal values, RBC (8.55 $\times 10^6$ cells/mL); WBC (7.81 $\times 10^3$ cells/mL); LYM (8.00 $\times 10^3$ cells/mL); HGB (14.50 g/dL); PCV (41.12%); MCH (16.98 pg); MCV (48.27 fL); PLT (374.2 $\times 10^3$ platelets/mL) LYM also decreased in (G2) to (7.04 $\times 10^3$ cells/mL) while MCV slightly increased (49.55 fL) . Mannan treatment demonstrated dose-dependent improvement (G3) showed limited effects whereas (G4) showed significant improvement , The best response was observed in (G5), with increased RBC (9.41 $\times 10^6$ cells/mL), LYM (9.065 $\times 10^3$ cells/mL), and HGB (14.91 g/dL), along with reduced PLT (336.2 $\times 10^3$ platelets/mL) and near-normal MCV (48.35 fL). G6 showed moderate improvement, while atorvastatin (G7) produced partial recovery. (G8) remained within physiological limits. These findings indicate that mannan exerts significant regulatory effects on hematological parameters, with optimal efficacy at moderate doses, supporting its potential as a functional dietary supplement in managing metabolic disorders.

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Keywords: Mannan, High-fat diet, Hematological parameters, Rattus norvegicus, Immune response, Metabolic stress

1 Introduction

Metabolic disorders particularly hyperlipidemia have become a major global health concern due to changes in dietary habits and reduced physical activity these disorders extend beyond dyslipidemia and are associated with a wide range of physiological alterations, including changes in blood composition and immune function , which increase the risk of cardiovascular diseases and metabolic liver disorders [1]. high-fat diets have been shown to induce a state of low-grade chronic inflammation reflected in altered blood biomarkers as a result of metabolic stress affecting hematopoiesis and blood cell function [2,3].

Hematological parameters such as white blood cell count (WBC) and lymphocytes (LYM) are key indicators of immune and inflammatory status, while red blood cell (RBC), hemoglobin (HGB), and packed cell volume (PCV) reflect oxygen transport capacity and hematopoietic activity, platelets (PLT) along with erythrocyte indices including mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) serve as reliable indicators of hematological balance and alterations associated with inflammatory and metabolic conditions. Previous studies have reported that hight-fat diets can disrupt these parameters through mechanisms involving chronic inflammation, oxidative stress and impaired iron metabolism [4,5].

Polysaccharides are bioactive compounds known for their significant roles in regulating metabolism, modulating the gut microbiota, and influencing the immune response [6]. Among these mannan- an essential component of yeast cell walls-has attracted considerable attention due to its biological activities which depend on its molecular structure [7]. mannan and its derivatives such as mannan oligosaccharides (MOS) have been reported to reduce inflammation and enhance immune balance through modulation of signaling pathways such as TLR4/NF-κB [8]. Mannan is wifely present in the cell walls of microorganisms, including yeasts, bacteria, and fungi. Notably *S. boulardii* is one of the most commonly used yeast strains in the food and medical applications due to its well-established probiotic and functional properties [9]. However studies examining the effect of isolated mannan on blood and physiological parameters associated with hyperlipidemia remain limited, particularly regarding its impact on blood components and the immune

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response under conditions of metabolic stress.

Accordingly, the present study aimed to evaluate the effect of mannan extracted from the cell wall of *S. boulardii* when administered orally to rats with hyperlipidemia induced by a high-fat diet, by examining its effect on hematological parameters including WBC, LYM, RBC, HGB, PCV, PLT, MCV, and MCH, to explore its potential role as a natural immunomodulatory compound and hematopoietic enhancer under conditions of metabolic stress.

2 Material and Methods

1-2 Experimental Animals

This study used Fourty-eight male albino rats of the Wistar strain (Rattus norvegicus), and the animals were subjected to experimental treatments that included oral administration of mannan previously extracted from the cell wall of *S. boulardii* at different doses according to the study's experimental design. The animals were 10 weeks old with an initial weight ranging from 120–150 g and were housed under standard laboratory conditions at a temperature of 22±2°C with a 12-hour light/12-hour dark cycle, with water and feed provided ad libitum. All procedures were conducted in accordance with the Guide for the Care and Use of Laboratory Animals [10]., adhering to approved ethical standards.

2-2 Experimental Design

It was decided to use 4 rats per group, with outliers excluded at a rate of 2 rats per group, the animals were randomly assigned to eight groups (n = 4 per group) according to a completely randomized design (CRD). Hyperlipidemia was induced by feeding the animals a high-fat diet for one month prior to the start of the therapeutic intervention, which is a well-established model for studying lipid metabolism disorders in rats [11,12].

The groups were distributed as follows:

G1: Standard diet + 0.9% physiological saline (NaCl) dosing (negative control)
G2: High-fat diet (positive control).
G3: High-fat diet + 25 mg/kg mannan
G4: High-fat diet +50 mg/kg mannan
G5: High-fat diet +75 mg/kg mannan
G6: High-fat diet + 100 mg/kg mannan
G7: High-fat diet+ 10 mg/kg atorvastatin
G8: Standard diet + 100 mg/kg mannan

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Treatment with mannan or atorvastatin began in the second month, while participants continued to follow the diet assigned to their respective groups; the intervention period lasted four weeks. Mannan isolated from the cell wall of *S. boulardii* was administered orally via a gastric tube daily at doses determined by body weight.Atorvastatin was administered in the same manner as a therapeutic control group, and the first group was given a saline solution.

3-2 Physiological analyses

Blood samples were collected at the end of the experimental period after an overnight fast. To alleviate pain, the rats were injected intramuscularly with 0.25 mg/100 g body weight of xylazine and 5 mg/ 100 g. Using 10-ml medical syringes, blood samples were drawn via cardiac puncture from all groups. To measure physiological parameters, blood samples were transferred to tubes containing an anticoagulant, while the remaining samples were transferred to test tubes without anticoagulant and left for 10–15 minutes at laboratory temperature. The samples were then centrifuged at 3000 rpm to separate the serum from the other components. The serum was separated and placed in tubes for physiological testing [13].

1.3.2 White Blood Cell (WBC) Count

The total white blood cell count was measured using Turk's solution The solution was prepared by mixing 2 mL of glacial acetic acid with 98 mL of distilled water, then adding one drop of methylene blue as a color indicator. The test was conducted by placing 0.4 mL of the Turk's solution into test tubes, followed by the addition of 0. 02 mL of drawn blood. The tubes were then shaken well and transferred to a white blood cell counter. A drop was taken, a slide cover was placed over it, and it was left for 2 minutes to allow the cells to settle. It was then examined at 10x magnification, and the white blood cell count was calculated according to the method [14]. using the following equation:

W.B.C/mm3=Number cells ×50

2-3-2 Red Blood Cell Count (RBC)

1-2-3-2 Total red blood cell count (RBC)

The blood cell counting method and Hayme's solution were used. The solution was prepared by dissolving 0.5 g of NaCl sodium chloride and 0.5 g of $Na_2\ SO_4$ sodium sulfate and 0.25 g of mercury chloride $HgCl_2$ in 100 mL of distilled water [15]. This served as a diluent for red

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blood cell counting, and the test was performed by taking 2 mL of the Himes solution into a test tube and adding 0.02 mL of drawn blood, the mixture was shaken well, a drop of the mixture was taken and transferred to a hemocytometer, a coverslip was placed over it, and it was left for two minutes to allow the cells to settle The hemocytometer was then placed under a microscope and examined at a magnification of x40, and the red blood cells in five small squares using the following formula:

R.B.CS/mm3= Number R.B.CS counted x 10000

2-2-3-2 Hemoglobin Estimation HGB

A hemoglobin meter and Drabkin's solution were used. The solution was prepared by dissolving 1g of sodium bicarbonate and 0.05g of potassium cyanide and 2g of potassium ferricyanide in 1 liter of distilled water as a dilution solution to determine the hemoglobin concentration in the blood sample [15]. The test was performed according to the following steps:

5 mL of Darbeken solution was placed in a clean test tube, to which 0. 02 mL of the drawn blood. The tube was shaken well and then left for 10 minutes. The hemoglobin meter was then zeroed with distilled water, and the tube was placed in the device. The HGB value appeared on the device's screen in units of g/dL [16].

3-2-3-2 Packed cell Volume Measurement P.C.V

Capillary tubes, a microcentrifuge, and a hematocrit reader were used to measure the hematocrit percentage [15,16]. The testing procedure was carried out according to the following steps:

Blood was allowed to flow into the capillary tube via capillary action, leaving approximately 15 mm of the tube unfilled. One end of the tube was then sealed with synthetic clay, and the tube was placed in the microcentrifuge and spun for 5 minutes at 11,000 rpm. The tubes were then removed from the microcentrifuge, and the packed cell volume (PCV) was read using a hematocrit reader.

4-2-3-2 Red Blood Corpuscles Calculation

Red blood cell indices were calculated using values obtained from packed cell volume, hemoglobin concentration, and total red blood cell count [15], according to the following equations:

A- Mean Corpuscular Hemoglobin (M.C.H)

This value represents the mean corpuscular hemoglobin

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concentration of the sample's red blood cells; it is measured in picograms and calculated using the following equation**:**

$$\mathbf{M.C.H_{(PG)}} = \frac{\boldsymbol{Hemoglobin\ in\ gm/d}}{\boldsymbol{Red\ cells\ count\ per\ liter}} \times \mathbf{10^3}$$

B- Mean corpuscular volum (M.C.V)

This value represents the volume of a red blood cell, measured in femtoliters (FL), and is calculated using the following equation:

$$\mathbf{M.C.V_{(FL)}} = \frac{\boldsymbol{Packed\ cell\ Volum}}{\boldsymbol{Red\ cells\ count\ per\ liter}} \times \mathbf{10^{15}}$$

3-3-2 Measuring the lymphocyte count (LYM)

The method described by [17] was used to measure the lymphocyte count using the Ficoll-Isopaque (FI) method, 2 mL of blood serum was diluted in 5 mL of a 2% PRS-FCS-0.1% NaN solution, and the diluted blood was carefully added to 3 mL of rat cell-calibrated FI solution (density = 1.09 × 10^3 g/mL) . After centrifugation, the cells were washed three times and resuspended in 4 mL of PBS–FCS–NaN solution. Prior to staining, the viability of each lymphocyte suspension was assessed using acridine orange – ethidium bromide staining. Mononuclear cells were incubated in 50 µL of secondary antibody for 20 minutes at 4°C. The cells were washed in PBS – FCS-NaN, then 50 µL of an appropriate dilution of the secondary antibody, as determined by titration, was added. After 20 minutes at 4°C, then the cells were washed with PBS and resuspended in 150 µL of PBS and immediately analyzed by flow cytometry.

4-3-2 Total Platelets Count PLT

The experiment was conducted as described in [15], using a blood cell counter and ammonium oxalate solution. This solution was prepared by dissolving 1 g of ammonium oxalate in 100 mL of distilled water. The test was performed by placing 0.38 mL of the ammonium oxalate solution into a clean test tube, then adding 0. 02 mL of the drawn blood and shaking the mixture well. A drop of the mixture was then transferred to a hemocytometer. After placing a cover slip on the slide and allowing it to stand for 15 minutes to allow the cells to settle, the hemocytometer was placed under a microscope and examined at a magnification of x40. The platelet count was calculated by counting small squares in the large central square and according to the formula:

Platelet /mm3= Number of cells counted × 100

ISSN 2072-3857

5-3-2 Statistical Analysis

The data were statistically analyzed using GenStat software, where a one-way analysis of variance(ANOVA)was performed using a completely randomized design (CRD).When significant differences were found between treatments, Duncan's Multiple Range Test was used to compare means at a significance level of $P \leq 0.05$, as recommended for the analysis of data from biological experiments [18].

3 Results and Discussion

Tables 1 and 2 shows the effect of mannan isolated from the cell wall of *S. boulardii* at different doses on blood parameters in rats with diet-induced hyperlipidemia. These parameters included (WBC, RBC, LYM, HGB, PLT) as well as red blood cell indices (PCV, MCV, MCH). The values are presented as means for each experimental group following the completion of the four-week treatment period

Table 1. Effect of mannan extracted from the cell wall of *S. boulardii* on blood cell parameters in rats

Group	WBC	RBC	LYM	HGB	PLT
G1(control-)	7.81 a	8.55 b	8.00 cd	14.50 cd	374.2 bc
G2 (Control+)	10.86 d	7.79 ab	7.04 b	9.79 a	395.8 bc
G3 (25 mg/kg)	10.08 d	7.95 ab	7.00 b	12.30 b	397.2 c
G4(50 mg/kg)	9.04 c	8.14 ab	8.43 de	14.58 cd	368.5 abc
G5(75 mg/kg)	8.04 ab	9.41 c	9.06 e	14.91 d	336.2 a
G6(100 mg/kg)	8.91 bc	8.44 b	7.57 bc	13.14 b	361.0 abc
G7(Atorvastatin)	7.84 a	7.48 a	6.12 a	13.48 bc	376.2 bc
G8(100mg/kg+normal Diet)	8.11 ab	7.33 a	7.96 cd	13.05 b	358.8 ab

* Different letters within the same column indicate significant differences at the $P < 0.05$ level, as determined by Duncan's multiple range test.

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Table 2 Effect of mannan extracted from *S. boulardii* yeast on red blood cell volume

Group	PCV	MCH	MCV
G1(control-)	41.12 bc	16.98 bc	48.27 a
G2 (Control+)	38.52 a	12.50 a	49.55 a
G3 (25 mg/kg)	45.05 d	15.40 b	56.62 b
G4(50 mg/kg)	42.50 c	17.98 c	52.52 ab
G5(75 mg/kg)	42.92 cd	15.77 b	48.35 a
G6(100 mg/kg)	41.40 bc	15.60 b	49.23 a
G7(Atorvastatin)	39.50 ab	17.77 c	52.60 ab
G8(100mg/kg+normal Diet)	38.55 a	17.98 c	56.75 ab

and hemoglobin indices in rats

* Different letters within the same column indicate significant differences at the $P < 0.05$ level, as determined by Duncan's multiple range test.

The results shown in Tables 1 and 2 indicate the effect of mannan extracted from the cell wall of *S. boulardii* at various doses. The results showed that the standard feed group (G1) yielded normal white blood cell counts (WBC) reaching 7.81×10^3 cells/mL,reflecting physiological stability in the absence of any metabolic stress. In contrast,the untreated high-fat diet group(G2) recorded a clear and significant increase of 10.86×10^3 cells/mL, indicating the stimulation of a systemic inflammatory response attributed to increased intestinal permeability and the transfer of endotoxins such as LPS into the bloodstream, leading to the activation of immune cells and an increase in white blood cell count [19,20].Meanwhile, group (G3) showed no significant difference from (G2), recording 10.08×10^3 cells/mL, indicating that the low dose of mannan was insufficient to suppress the inflammatory response On the other hand, the groups treated with higher doses of mannan (G4–G6) showed a

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gradual decrease in WBC, with values of 9.04×10^3 (G4) and 8.04×10^3 (G5) - 8.04×10^3 and (G6) $-10^3 \times 8.91$ cells/mL, approaching (G5) to the control group, indicating the effectiveness of the medium dose in regulating the immune response and reducing inflammation. This effect is attributed to the immunomodulatory role of mannan as a modulator of the inflammatory response by improving microbiota balance and reducing the activation of LPS-associated inflammatory pathways [21,22]. The atorvastatin group (G7) showed a clear decrease to 7.84×10^3 cells/mL with no significant difference from the control, reflecting its anti-inflammatory effect and reduction of leukocyte recruitment to sites of inflammation [20]. Meanwhile, (G8) recorded 8.11×10^3cells/mL,which is within near-normal limits, suggesting that mannan does not stimulate an inflammatory response under physiological conditions but acts as an immunomodulator whose effect depends on the presence of a pathogenic agent or inflammatory stress.

As for the RBC results shown in Table (1-3), they indicated that the control (G1) had a normal red blood cell count of 8.55×10^6 cells/mL, whereas this value decreased in the high-fat diet group (G2) to 7.79×10^6 cells/mL, indicating a negative effect of the diet on red blood cell formation, which is attributed to chronic inflammation and iron metabolism disorders associated with the metabolic state, as documented in models of obesity and nutritional stress [23,24]. (G3) showed no clear improvement(7.95×10^6 cells/mL) indicating the limited effect of the low dose of mannan, while (G4) showed a relative improvement of 8.14×10^6 cells/mL, reflecting the onset of the therapeutic effect. In addition (G5) recorded the highest value of 9.41×10^6 cells/mL, outperforming all other groups, indicating that the medium dose of mannan was the most effective in promoting red blood cell formation by improving nutrient absorption and reducing oxidative stress and inflammation. As for (G6) showed a good improvement of 8.44×10^6 cells/mL, but this was lower than (G5), confirming a dose-dependent response where optimal efficacy lies within a specific range [7]. On the other hand, the atorvastatin group (G7) recorded a value of 7.48×10^6 cells/mL with limited improvement compared to mannan, while group (G8) recorded the lowest value of 7.33×10^6 cells/mL mL, although it remained within physiological limits, which may reflect natural variation or a different regulatory effect in the absence of inflammatory stress. This is explained by the need to correlate RBC results with other indices such as HGB, PCV, MCV, and MCH to obtain a comprehensive physiological interpretation [24].

As for lymphocytes (LYM), the study results showed that the control group (G1) had a normal lymphocyte count of 8.00×10^3 cells/mL, whereas this value

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decreased in the high-fat diet group (G2) to 7.04 × 10^3cells/mL, indicating that adaptive immunity was negatively affected as a result of metabolic stress and low-grade inflammation associated with this diet, which leads to disruption of the gut-immune axis and altered distribution of immune cells, including lymphocytes [25,26]. Meanwhile (G3) showed no significant improvement (7.00×10^3 cells/mL), indicating that the low dose of mannan is insufficient to induce an effective immune response (Table 1-3).

In addition, the results for (G4) showed a rise to 8.43×10^3cells/mL, reflecting the onset of an immune response, while Group (G5) a higher value of 9.06 × 10^3 cells/mL, indicating clear stimulation of adaptive immunity This is attributed to the role of mannan as an immunomodulator that enhances lymphocyte activity by improving microbiota balance and reducing inflammation [27] . As for (G6) it showed a limited improvement of 7.57 × 10^3 cells/mL, indicating a dose-dependent response, with optimal efficacy observed at moderate doses, a finding supported by studies suggesting that the effects of mannan and prebiotics are regulatory rather than linear [28].

On the other hand, the atorvastatin (G7) recorded the lowest value of 6.12 × 10^3 cells/mL, which may reflect a relatively inhibitory effect on certain components of adaptive immunity or a redistribution of lymphocytes in the context of reduced systemic inflammation, whereas the (G8) remained close to the control group, suggesting that mannan, under physiological conditions, does not cause excessive activation of the immune system but acts as an immunomodulator whose effect depends on the presence of an inflammatory challenge [29].

On the other hand, the results of the study regarding hemoglobin (HGB) and packed cell volume (PCV), as shown in Tables (1 and 2) revealed a clear decrease in the high-fat diet group (G2) with a hemoglobin concentration of 9.79 g/dL compared to the control group (G1) which recorded 14.50 g/dL. The PCV value also decreased to 38.52% compared to 41.12% in the control group. These results indicate a disturbance in erythropoiesis attributed to the inflammatory effects and oxidative stress associated with the metabolic state resulting from the high-fat diet, whereas the group treated with a low dose of mannan (G3) showed partial improvement, with HGB rising to 12.30 g/dL and PCV to 45.05%, indicating the limited effect of the low dose despite an initial positive response, while groups (G4) and (G5) recorded the best values, with HGB in group (G4) reaching 14.58 g/dL and PCV 42.50%, while group (G5) HGB of 14.91 g/dL and PCV of 42.92%, values that approached or exceeded those of the control group, reflecting a marked improvement in hematopoietic efficiency and the restoration of physiological balance. (G6) showed moderate improvement, with HGB at

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13.14 g/dL and PCV at 41.40%, supporting the presence of a dose-dependent response, with optimal efficacy observed at moderate doses compared to higher doses.

In addition, the atorvastatin (G7) showed partial improvement, with HGB at 13.48 g/dL and PCV at 39.50% , indicating its role in reducing inflammatory effects without achieving the same efficacy as mannan in supporting red blood cell formation, while group (G8) remained within normal physiological limits, with HGB at 13.05 g/dL and PCV at 38.55% without causing excessive changes, indicating that mannan acts more as a regulator of blood parameters than as a direct stimulator.

These findings are consistent with recent studies indicating that obesity and chronic inflammation negatively affect red blood cell parameters, whereas reducing inflammation and improving the microenvironment can contribute to the gradual restoration of HGB and PCV to normal levels [8,23,24].

In contrast, the study results for mean corpuscular hemoglobin (MCH) and mean corpuscular volume (MCV), as shown in Table(2) a clear decrease in the MCH value in the high-fat diet group (G2) where it reached 12.50pg, compared to the control group (G1) which recorded 16. 98 pg, indicating a decrease in hemoglobin content in red blood cells, which reflects a negative impact of metabolic disorders and chronic inflammation resulting from the high-fat diet, whereas the groups treated with mannan showed a marked improvement in this indicator, as MCH in (G3)to15.40 pg, while (G4) recorded the highest value of 17.98 pg, followed by (G5) with a value of 15.77 pg, as well as (G6) which recorded 15.60 pg, while (G8) also recorded a high value of 17.98 pg. These results indicate improved red blood cell formation efficiency and increased hemoglobin content,and possibly improved absorption of the elements necessary for hemoglobin synthesis under the influence of mannan, especially at medium doses.

As for the mean corpuscular volume (MCV), it increased in the high-fat diet group (G2) to 49.55 fL compared to 48.27 fL in the control group (G1), indicating a disturbance in red blood cell volume. The (G3) recorded the highest increase in this parameter, reaching 56.62 fL, reflecting a clear abnormality in red blood cell maturation or a compensatory response to metabolic stress, whereas MCV remained close to normal limits in some treatment groups, reaching 48. 35 fL and in (G6) 49.23 fL, values close to those of the control group, indicating an improvement in red blood cell size uniformity. On the other hand, group (G4) recorded a value of 52.52 fL, while in the atorvastatin group it reached 52.60 fL in (G7), which are values higher than the control and indicate partial improvement with some remaining irregularity. As for (G8) it

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recorded the highest value of 56.75fL, indicating continued variation in red blood cell size despite the absence of a high-fat diet. Collectively, these results suggest that a high-fat diet leads to qualitative changes in red blood cell indices, manifested as a decrease in MCH and an increase in MCV, which is consistent with the effect of inflammation B and oxidative stress on red blood cell formation and function. In contrast, treatment with mannan helped restore these indices to normal levels, particularly at moderate doses, supporting its role as a bioactive compound that improves immune balance and metabolism These findings are consistent with the observations of [23]. regarding the association between metabolic disorders and reduced red blood cell indices, as well as the importance of interpreting these values within the reference ranges associated with physiological status, as explained by [24].

Similarly the results of the platelet (PLT) study, shown in Table (1) revealed a clear increase in platelet count in the high-fat diet group (G2), reaching 395.8×10^3 platelets/mL compared to the control group (G1)which recorded 374.2×10^3 platelets/mL, suggesting a concomitant inflammatory state and platelet activation associated with metabolic stress.The group treated with the low dose of mannan (G3) recorded the highest platelet count of 397.2×10^3 platelets/mL, indicating that this dose was insufficient to reduce inflammatory activity; rather, the platelets remained in a state of activation elevated; this increase is attributed to the fact that a high-fat diet promotes the production of free radicals and inflammatory mediators, leading to increased platelet activity and aggregation, as documented in models of obesity and high-fat diets [30,31].

In contrast, the (G5) recorded the lowest platelet count of 336.2×10^3 platelets/mL, indicating the ability of mannan at the medium dose (75mg/kg) to reduce inflammatory activity and restore platelet function. Similarly, group (G4) recorded a value of 368.5×10^3 platelets/mL, and group (G6) recorded 361.0×10^3platelets/mL, which are lower than those of the high-fat diet group and indicate a dose-dependent relative improvement.

As for the atorvastatin group (G7) it recorded a value of 376.2×10^3 platelets/mL, which is close to the control group, reflecting its effect in reducing the inflammatory state without reaching the hypoplatelet level achieved by the optimal dose of mannan, while (G8) recorded a value of 358.8×10^3 platelets/mL, which is within physiological limits, indicating that mannan under normal conditions does not lead to excessive platelet inhibition but rather regulates their activity.

In general, the remaining groups (G4, G6, G7,G8) showed moderate values with significant overlap, suggesting a partial regulatory effect dependent on dose and type of treatment, as reducing inflammation does not necessarily lead

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to complete inhibition of platelets but rather to a readjustment of their activity within physiological limits.These results are consistent with what recent studies have indicated that obesity and the Western diet are associated with platelet hyperactivation and functional changes in platelets, reflecting the close relationship between chronic inflammation and the platelet response [32,33].

4 Conclusion

The results of this study show that mannan isolated from the cell wall of *Saccharomyces boulardii* has a clear effect on improving the physiological parameters associated with blood disorders caused by a high-fat diet in rats. The high-fat diet led to noticeable changes characterized by an increase in white blood cells (WBC) and platelets (PLT), along with a decrease in red blood cells (RBC), hemoglobin (HGB), packed cell volume (PCV), and mean corpuscular hemoglobin (MCH), as well as a disturbance in mean corpuscular volume (MCV), confirming a state of metabolic stress accompanied by an inflammatory response and impaired hematopoietic efficiency. Treatment with mannan showed a clear improvement in most hematological parameters compared to the group fed the untreated high-fat diet, and this effect was dose-dependent within a specific range. The 75mg/kg produced the best hematological response, leading to increases in RBC,HGB, and PCV, an improvement in MCH, and a return of MCV toward normal limits, along with a marked decrease in WBC and PLT, indicating improved erythropoiesis and reduced inflammation. In contrast, the higher dose (100 mg/kg) did not show any clear additional improvement compared to the medium dose, suggesting the existence of an optimal dose range for the biological response. The atorvastatin group also showed partial improvement in some parameters, particularly in reducing WBC; however, its effect on improving red blood cell parameters was less effective compared to mannan, reflecting the difference in mechanism of action between drug therapy and natural bioactive compounds. Administration of mannan at a dose of 100 mg/kg with the standard diet maintained hematological values within normal limits without causing adverse changes, indicating the compound's safety and lack of harmful effects on normal physiological conditions. Taken together, these results suggest that mannan extracted from *S.boulardii* possesses promising hematological and immunological properties in improving blood parameters and mitigating the adverse effects of a high-fat diet, particularly by reducing inflammation and improving hematopoietic efficiency, with optimal efficacy observed at moderate doses. However, future studies to elucidate the molecular mechanisms underlying its effects on hematopoiesis and immune response

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regulation will be necessary for a deeper understanding of its biological role.

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