Extracting β-glucan from Saccharomyces cerevisiae,and its biological effect in reducing cholesterol levels in the laboratory and studying its effect

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Abstract

Glucan was extracted from acid-alkaline-based yeast samples Hydrolysis method; the dry weight of glucan was 4.8g/100g of Yeast. Carbohydrate and protein contents were determined for the glucan sample and the results indicated that the carbohydrate and protein ratio in the glucan was 44% and 0.45% ,respectively. Glucan A analyzed by FT-IR and the results confirmed that the extracted glucan showed a high degree of similarity and purity compared to the standard and the extracted glucan was considered β glucan examination of the compound extracted by FTIR in the diagnosis of some major groups involved in the composition of the compound ,and the peak of Absorption at 1074 cm-1 and peaks at 1156-1165 cm-1 which is the hallmark of the β-glucan structure that extends with the standard 1051 cm-1 indicates the linear structure of β-glucan and the compound was also examined by ultraviolet light to estimate the carbohydrate and protein contents .The results indicated the the ratio A Carbohydrate and protein of the extracted glucan were 44 %and 0.45% respectively as components of both glucose and protein A important indicators of the purity of glucan and a high amount of sugars with of protein content and compare the the the standard compound and the results showed that the compound extracted matches the standard complex .The ability of the compound extracted in of concentrations (10 mg/body weight and 40 m / body weight) to lower blood lipid levels was tested in the laboratory ,where the concentration of 10 mg/body weight extracted from the yeast S.cerevisiae showed a significant effect in lowering the ratio of cholesterol, where the percentage of decrease was (123) compared to positive control therapy (mg/dl 262) in contrast to the concentration of 40mg / bw ,where cholesterol decreased by (184.7 mg/dl) compared to positive control factor..

Keyword: Extract, β-glucan, cholesterol, *S. cerevisiae*, acid-alkaline

Introduction

β-glucan is one of the important natural substances due to its biological activity, immune and healthy reactions and when applied in different systems in vivo and in vitro (1) β-glucan is a complex polysaccharide found in the cell wall of organisms from various sources, including algae, which are single-celled organisms It carries out photosynthesis (2, 3). Yeasts like *S. cerevisiae* (4), (5), plants such as oats (6), and barley (7).

Yeast *S. cerevisiae* is a fungus commonly used in baking and fermentation. It contains a unique type of carbohydrate known as β-glucan, which are long chains of glucose molecules bonded together. This glucan has many health benefits, including boosting the immune system, lowering cholesterol levels, and improving gut health. β-glucan found in yeast is particularly effective in stimulating the immune system. This is done by activating certain cells in the body, especially connective tissue, which are responsible for the

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identification and destruction of harmful pathogens. This activation increases the production of cytokines, molecules that indicate that they help regulate the immune response β Glucan yeast is produced in copious quantities and is considered safe to consume without side effects.

S. cerevisiae yeast has a thick cell wall composed of sugars and proteins that protect the internal parts of the cell (8). The cell wall consists of up to 55% glucan β (1-3) connected and 12% glucan β (1-6) (8). Yeast-derived β-glucan has a linear backbone of 1-3 D-glucose (β), connected by side chains (1-6 β,) of different lengths. Figure 8 shows the chemical composition of the compound.
Yeast fungus consists of β-glucan on its cell wall because most of its cell walls are made up of β-glucan. The S. cerevisiae cell wall contains proteins bound to glucose components such as glycoproteins and proteins and contains aphids, chitin, β-1,3-glucans and β-1,6-glucans that have the function of strengthening cell structure and as food stores (9)

3- Materials and methods

3-1 Preparation and activation of yeast *S. Cerevisiae*

Five grams of yeast was cultured in the medium of SDA (Sabouraud dextrose broth) and incubated in a vibrating incubator for two days. After the end of the incubation period, a swab was taken from the medium and distributed on a plate to ensure the effectiveness of the yeast.

3-2 Glucan β extract from *S. cerevisiae*

S. cerevisiae yeast from Angel Yeast was obtained from domestic markets. Yeast was prepared and the extraction process took place as reported in (10). 30 g of yeast was mixed with 5times the amount of pre-prepared sodium hydroxide solution, the mixture was heated at 80 ° C with continuous magnetic stirring for two hours and then centrifuged at 6000 cycles / 15 minutes at 4 °C. To separate the outer membrane of yeast. The precipitate was washed twice in distilled water, followed by 5 ml of acetic acid (CH_3COOH) 0.5N to the precipitate, with continuous magnetic stirring at a temperature of 80 ° C for two hours. It was then centrifuged at 6,000 cycles / 15 minutes, then the precipitate was washed with distilled water twice and dried in a convection oven at 60 ° C for 48 hours. Absorption was measured at 480 nm using a spectrophotometer, and the product concentration was measured based on the standard curve.

3-3 Laboratory feeding with β-glucan extract and studying its health effects

After the approval of the Ethical Committee to conduct animal studies at the University of Kufa and the experimental protocol, all animals (mice) were taken care of in the animal house of the Faculty of Science - University of Kufa according to the instructions of the Animal Welfare Council as shown in the annex to the care guide No. (10) The experiments were 15 male mice, 60 days old, weighing (180 to 200 g), placed in special breeding cages in a clean, environmentally controlled room with a temperature of (2±20) ° C over 24hours. The mice received water and a diet. allocated for them for two weeks to adapt before the start of the experiment.

3-4 Diet

Adaptation of the high-fat diet Based on the formula given by Levin and Dan Meynell According to (11) the high-fat diet shown in Table (3-4) all components of the high-fat diet were well mixed. The stomachs of mice are high in fat, causing high cholesterol for 4 weeks.

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Table (1-1). Shows the normal regimen and the high-fat system and their components(12).

Natural normal diet		**High-fat diet**	
100% sadness	Nutrients	100% sadness	**Nutrients**
48.8	Carbohydrate	43	**Carbohydrate**
21	protein	17	**protein**
3	fat	40	**fat**
Ingredients 100g/g Natural Normal Diet		**Ingredients of 100g/g High Fat Diet**	
0.8	Calcium	68.0	**Crushed rat feed**
0.4	Phosphorus	6.0	**oil**
5	Fibers	6.0	**Call**
13	Moisture content	20.0	**Milk powder**
8	ashes		
306.2	Total Power)cal / 100 g(	414.0	**Total Power)kcal / 100 depression(**

3-5 Animal Study Design:

The weight and body mass index of the group of rats on a high-fat diet were compared with the control group, the negative control of the rats after 4 weeks of induction of obesity, and the body weight and BMI of the obese were considered obese (13). The rats were dosed orally with the compound every day for a month.

Table (1-2). Shows the experimental design of rat groups.

Group	Treatment
first group	**Control diet for standard (-) negative control rats (normally fed rats)**
second group	**Standard positive (+) cholesterol control (high-cholesterol diet fed rats)**
third group	**Positive control use of Atorvastatin (10 m/kg)**
Fourth group	**β-glucan extract from S. cerevisiae (10mg/Bw)**
Fifth group	**β-glucan extract from S. cerevisiae (40mg/Bw)**

3-6 Collection of blood samples

The rat was injected intramuscularly with (0.25 ml xylazine / 100 g body weight and 5 mg ketamine/100g) to relieve and reduce any pain. Blood samples were taken, as blood samples were drawn from the heart of all groups used in the experiment using sterile medical syringes with a capacity of 10 ml. From the blood, the samples were transferred to tubes containing an anticoagulant substance for the purpose of measuring physiological

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parameters, while the other part of the blood was placed in special tubes devoid of any anticoagulant substance and left at the laboratory temperature for 10-15 minutes. The blood serum is separated from the rest of its components and placed in test tubes.

4- Results and Discussion

4-1 β-glucan extract from *S. cerevisiae* by Alkaline-acid method

β-glucan compound was obtained from *S. cerevisiae* yeast and was about five grams. The β-glucan gel was of a mucilaginous consistency, light creamy color, and tasteless, insoluble in water, while its powder, after drying at 60°C for 48 hours, had a brownish color using the alkane method. As this method was adopted by (13), the resulting compound was examined and verified with standard β-glucan using the FTIR test and photographic total sugar.

4-2 Effect of β-glucan on cholesterol level

The current results showed that β-glucan extracted from S. cerevisiae yeast had a significant effect on lowering cholesterol levels**.** ($F_{(4, 14)} = 44.91$, $P < .001$) β-glucan extracted from *S. cerevisiae* as the results showed, the concentration of 10 mg/ Bw extracted from the yeast S.cerevisiae gave a significant effect in reducing cholesterol, as the percentage of decrease was (123) compared to the positive control treatment, unlike the concentration of 40 mg/ Bw, as it reduced cholesterol by (184.7) compared to the positive control treatment (262). On the other hand, the concentration of 40 mg / Bw of the mixture of yeast and bacteria gave a significant effect in reducing the average cholesterol to (103.4) compared to the positive control treatment. On the contrary, it did not show a high efficacy in lowering cholesterol at a concentration of 10 mg/Bw of the mixture between the compound extracted from yeast and bacteria, as it reduced cholesterol by (144.7) compared to the positive control treatment. In a study conducted by (14) it was found that the level of total cholesterol decreased in the blood plasma of laboratory rats Sprague Dawley, as the level of decreased cholesterol was determined and measured using the enzymatic method. (14) indicated that crude β-glucan extracted from S. cerevisiae yeast at mg/bw10 concentration can lower total cholesterol levels to an extent close to the normal level of cholesterol in blood plasma and liver of S. Dawley rats. It was found that β-glucan It lowers blood cholesterol by increasing the viscosity of the gastrointestinal tract, thereby reducing, or affecting cholesterol absorption and bile acid reabsorption, as well as through other effects on cholesterol homeostasis (15) and (16). β-glucan can also reduce blood cholesterol by blocking the absorption of cholesterol from food in the stomach and intestines (17).

On the other hand, it was found that treating rats with atorvastatin, which acts as a catalyst in the formation of cholesterol, which is in the form of 3-hydroxy-s3-methyl-glutaryl-coenzyme A (HMG-CoA), did not significantly affect the reduction of cholesterol level compared to the control treatment. positivity (the rats were fed high-fat food as well as the rats were not dosed with β-glucan) as well as compared to the treatment of rats with β-glucan extracted from yeast at a concentration of 10 mg/dw.

4-3 The effect of β-glucan on the level of triglycerides

The current results showed that β-glucan extracted from *S. cerevisiae* had a significant effect on reducing the level of triglycerides ($F_{(4, 14)} = 23.80$, P< <.001). the concentration of 10 mg/Bw β-glucan had a significant effect on reducing triglycerides by (49.64) compared to the positive control treatment (162.3). On the other hand, the concentration of 40mg/Bw of the yeast compound was not highly effective in reducing triglycerides compared to the positive control treatment, as the percentage of reduction for fats was (91.28). In a clinical trial of 268 men and women with

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high cholesterol, oat beta glucans were found to lower cholesterol and triglycerides, thereby reducing the risk of cardiovascular disease (18). In a study conducted by (19), which aimed to determine whether consuming 6 g per day of barley beta-glucan could have a favorable effect on intestinal metabolism, it was found that β-glucan had a significant effect in improving the metabolism of barley. intestines and lowering cholesterol and triglycerides (19)On the other hand, it was found that low doses of β-glucan led to an increase in body weight, elevated levels of glucose in plasma and levels of triglycerides, while higher doses of β-glucan gave a beneficial and different effect from the effect of lower concentrations (20).

4-5 Effect of β-glucan on High-density Lipoprotein (HDL)

The current results showed that although there were significant differences between the treatments in increasing the rate of good fats (HDL) ($F_{(4,\ 14)} = 11.03$, $P< 0.001$), the β-glucan A extracted at a concentration of 40 mg/Bw from yeast. S. cerevisiae (65.63) had a significant effect like the positive control treatment (about 74), respectively, compared to the average of the negative control (68.46) with the positive comparison treatment, as shown in figure (1). (17) demonstrated that β-glucan reduces LDL cholesterol and increases HDL, which may alleviate insulin resistance (17). (21) indicated that the mechanism of action of β-glucan isolated from oats that would reduce LDL and TC triglyceride levels differs from that which may affect HDL. High-density lipoprotein (HDL) can be reduced due to several factors such as being overweight, excess saturated fat, and calorie intake, while other factors can lead to increased HDL levels, such as increased physical activity and consumption of unsaturated fats (22). These changes occur due to adaptations in lipid metabolism, which increases lipoprotein enzymatic activity, and thanks to greater hydrolysis of TG-rich lipoproteins, thus causes less formation of hardened LDL and increases serum concentrations of nascent HDL (23).

4-6 The effect of β-glucan on low-density lipoprotein (LDL)

The present results showed that β-glucan extracted from S. cerevisiae had a significant effect on reducing LDL ($F_{(4,\ 14)} = 11.03$, $P< 0.001$.) The β-glucan extracted from yeast at a concentration of 10 mg/Bw at a ratio of (43.41) had a normal effect compared to the negative control treatment (47.16) as well as the concentration of β-glucan extracted from yeast at a concentration of 40 mg / Bw with a ratio of (10.44) , as shown in figure (1).

4-7 Effect of β-glucan on Very Low-density Lipoprotein (VLDL)

The current results showed that β-glucan extracted S. cerevisiae had a significant effect on reducing VLDL. ($F_{(4,\ 14)} = 29.91$, $P<0.001$.) at a concentration of 10 mg/Bw ratio of (9.93) The showed that the rate of VLDL decreased compared to its rate (32.47) in the positive control treatment (control +), as shown in figure (1), while there is no significant difference and the rate of VLDL in the treatment of 40mg/Bw compared with a control sample of the drug.

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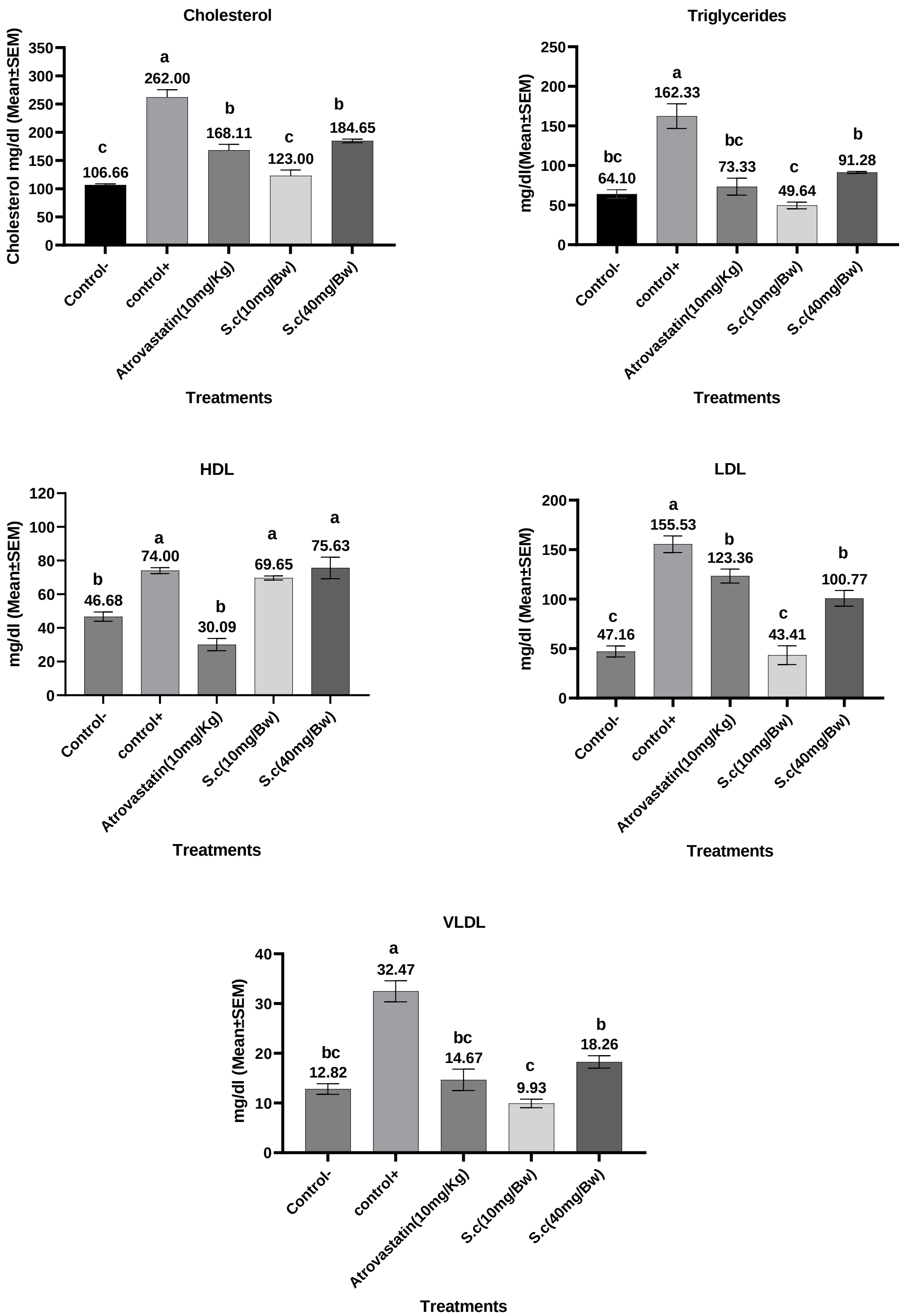

Figure 1:The level of lipids in the blood of rats after ingestion of β-glucan extracted from S.cerevisiae yeast and atorvastatin treatment for 30 days. The different letters indicate a significant difference at the 1% level of significance.

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4-8 Effect of β-glucan on white blood cells (WBCs)

Although the results of the current study showed that β-glucan extracted from yeast (*S. cerevisiae*) had an insignificant effect in reducing the rate of WBCs ($F_{(4, 14)}$ = 2.65, P< 0.096), the rate of white blood cells (WBCs) White blood (WBCs) decreased at a concentration of 10 mg/Bw of a β-glucan compound extracted from yeast to about (7.43) compared to an average of 14.53) with (Atorvastatin) treatment at a concentration of 10 mg/kg. On the other hand, there were no significant differences. In the rest of the treatments, a concentration of 40 mg/Bw reduced the rate of white blood cells (WBCs) compared to the positive control treatment (9.05) as in Figure (2). It was found that β-glucan can enhance the participation of white blood cells in the immune response, which increases Cell activity that improves phagocytic activity and cytokine secretion, which in turn stimulates the formation of new white blood cells. This may be the reason for the high rate of white blood cells. In a study conducted by Zhu on catfish, supplementation of at least V-containing compounds 25% of β-1,3/1,6 glucans in the feedstock can improve the phagocytic system through increased phagocytic activity (24). Biological factors modulating the immune response such as β1,3/1,6 glucans have the potential to be widely induced in organisms in both specific and nonspecific immune systems (25).

4-9 Effect of β-glucan in NEU

The current results showed that the β-glucan extracted from S. cerevisiae had a non-significant effect on the rate of Neutrophils ($F_{(4, 14)}$ = 3.62, P< 0.045). It was found that the rate of (NEU) increased in the treatment of yeast S. cerevisiae at a concentration of 10 mg/Bw at a rate of about (4.09), compared to a rate of (3.43) in the standard positive control treatment. On the other hand, it did not show a significant increase in the rate of NEU in the treatment of yeast S. cerevisiae at a concentration and at a concentration of 40 mg/Bw at a rate of (1.10). Compared to the positive control treatment (3.43) as in Figure (2).

4-10 Effect of β-glucan in Lymphocytes

The current results showed that the β-glucan extracted from S. cerevisiae had a non-significant effect on the rate of Neutrophils ($F_{(4, 14)}$ = 5.43, P 0.014). The β-glucan extract from S. cerevisiae yeast, at a concentration of 10 mg/Bw, showed a decrease in the rate of lymphocytes (Lym) at a rate of (5.56) compared to the control treatment of the drug (14.05).

4-11 Effect of β-glucan in Monocytes

The present results showed that β-glucan extracted from S. cerevisiae yeast and the mixture had a significant effect on the rate of monocytes ($F_{(4, 14)}$ = 8.86, P < 0.003). It was found that the rate of monocytes (MON) increased in the positive comparison treatment (Control) at a rate of about (1.24) compared to a rate of (0.0) in the treatment at a concentration of (40mg/bw) as in Figure (2). It was found that β-glucan acts as a cofactor, enhancing the immune response and increasing monocytes after vaccination (26), and in an animal study conducted by Wang et al., pigs showed a significant improvement in antibodies at 30 days after vaccination (27).

4-12 Effect of β-glucan on Hemoglobin

The current results showed that β-glucan extracted from S. cerevisiae had a significant effect on the hemoglobin rate ($F_{(4, 14)}$ = 5.31, P< 0.015). It was found that the hemoglobin rate was almost constant among all treatments, except for the positive control treatment (Control +), in which the hemoglobin rate decreased to about (6.83), , as shown in figure (2).The study (28) showed that the consumption of oat bran by diabetic patients did not cause a significant difference in hemoglobin levels. On the other hand, consumption of oat bran resulted in better control of weight gain in diabetic pregnant mothers and subsequently improved their BMI status (28).

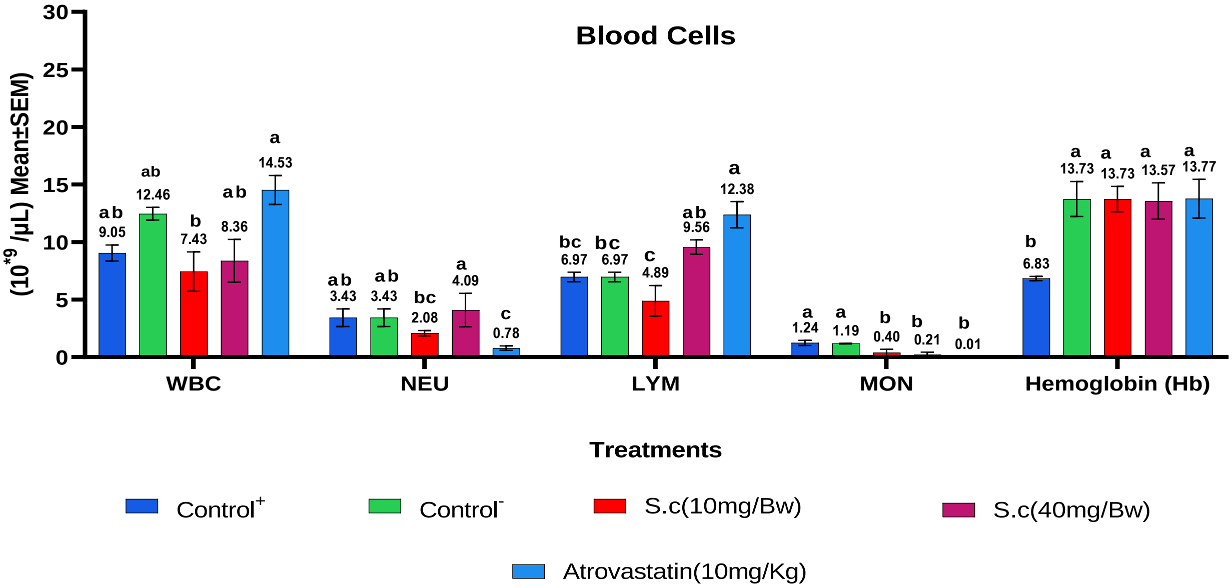

Figure 2: Blood cells in the rats after ingestion of β-glucan extracted from *S. cerevisiae* and atorvastatin treatment for 30 days. Different letters indicate a significant difference at the 1% level of significance.

Conclusion

Among the considered methods for beta-glucan isolating, the most promising is the use of standard alkaline-acid extraction method for cell disintegration. It gives the highest yield and significantly reduces the production time compared with other method

Studies have shown that yeast beta-glucan, obtained from baking yeast, can be successfully used as lowering cholesterol in conclusion, the results of our investigation

Demonstrated that highly purified, yeast-derived beta-glucan modify plasma cholesterol levels as well as the fact that these glucans also strongly stimulate monocytes and macrophages supports the hypothesis of the macrophage-cholesterol axis.

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