Effect of β-glucan from *Lactobacillus fermentum* on serum lipid profile in rats

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Abstract

The study aimed to extract the compound β-Glucan from the cell wall of *Lb. fermentum Lactobacillus fermentum* CECT5716 and study its effect in reducing cholesterol levels in the blood of laboratory animals (rats). The following results were obtained: Examination of the extracted compound by FTIR in the diagnosis of some major aggregates involved in the formation of the compound, peak absorption appeared at 1074 cm^{-1} and peaks at 1156-1165 cm^{-1} which is the defining feature of the structure of β-glucan extending with the standard 1051 cm^{-1} refers to the linear structure of β-glucan. UV was used to evaluate the compound to determine the amount of protein and carbohydrates in the substance. The results indicated that the percentage of carbohydrates and proteins extracted in glucan was 44% and 0.45% respectively. The components of glucose and protein gave an important indicator about the purity of glucan, containing a high number of sugars with a low content of proteins, and compared it to the standard compound Nutricost®, USA. The results showed that the extracted compound was identical to the standard compound when using FTIR and UV tests. In addition, it was noticed that the level of cholesterol decreased to (82.2) when the rats were treated with β-glucan extracted from L. b fermentum, where the treatment of the bacterium beta-glucan at a concentration of (mg / bw10) showed that the level of cholesterol in the blood approached the levels of normal cholesterol. While the level of cholesterol in the rats treated with the same bacterial beta-glucan at a concentration of (40 mg/ Bww) was about (163), and this may be attributed to the fact that the concentration (10 mg / Bw) was the ideal concentration in lowering the level of cholesterol. Triglycerides, LDL, and raising beneficial fats (HDL). The product has also been shown to be effective in raising beneficial animal blood cells and immune cells, stabilizing red blood cells, and reducing white blood cells.

Keywords: β-Glucan, *Lactobacillus fermentum* , Extraction, cholesterol, LDL.

Introduction

Glucan is a homogeneous polysaccharide consisting of linear or branched chain β-D-glucopyranosyl glucose units with varying degrees of branching, interstitial in triple or single helix or randomly coiled. Different types of bonds (β 1-3) Different types of bonds (β 1-4) and (β 1-6) (9). The consumption of probiotics is a practical approach to modifying the intestinal flora and maintaining human health, Bifidobacterium, and Lactobacillus bacteria, are natural components of colon microbes and are the most commonly used probiotics in many foods and supplements (15 and 26). Several types of bacteria are naturally present in fermented foods and dairy products such as *Lb. planetarium Lb. fermentum, Lb.* Reuters 23*Lb. fermentum* bacteria isolated from breast milk, can inhibit pathogens, reduce cholesterol synthesis, and alter gut microbes (27and 23) β-glucan can also be isolated from different microorganisms such as bacteria that can produce diverse types of sugars called polysaccharides. Polysaccharides contain monosaccharides or several different monosaccharides (heterogeneous or homogeneous polysaccharides). Most of these sugars have a bacterial origin and are widely used in food and industrial applications (4 and 28).

Material and Methods

L. fermentum CECT5716 was obtained from Klaire Labs®, USA. The bacterial suspension was prepared by emptying the contents of a 30mg capsule containing *Lb. fermentum* CECT571 in a lyophilized form into test tubes on 100ml of MRS broth medium. The tubes were incubated under anaerobic conditions at a temperature of 37°C for 48hours for the purpose of activation, then inoculated from this growth in MRS broth at a temperature of 37°C under anaerobic conditions for 24 hours. β-glucan was extracted as mentioned in Tallon (24) and as followea: The bacterial suspension was centrifuged at 15,000 r/min for 15 minutes at 4°C. The extract was then washed with 5ml distilled water and then centrally expelled at 15,000 r/min for 15 min at 4°C. The viscous granules were suspended in a solution containing 5ml distilled water and 2g of NaCl and the extract solution was separated from the cells by an ultrasonic cleaner at 50w for 10 mins at a temperature of 4ºC. Samples were expelled at 6000 rpm for 30 mins at a temperature of 4°C to get rid of insoluble substances. The extract was deposited from the floating material by adding twice the amount of precipitate of cold ethanol for 24 hours and kept in the refrigerator at a temperature of 4ºC then centrifuged at 6000 rpm for 30 mins at a temperature of 4ºC. The granules containing the extract were suspended in 2ml distilled water and placed in a dialysis bag in a container containing 5L distilled water with continuous stirring at 25ºC for 48 hours and the distilled water was replaced at a rate of three times a day and then dried the extract in a hot air oven for 48 hours at a temperature of 15ºC and kept in sterile tubes until use.

The resulting compound was examined and confirmed with standard β-glucan using the FTIR test and photographic total sugar.

Feeding laboratory animals with β-glucan extract and studying its health effects

Animals and Diet

All animals (rats) were cared for in the Animal House of the College of Science - University of Kufa, adhering to the animal rules, after the ethical committee's clearance to perform animal experiments at the University of Kufa on the experimental protocol. The experimental animals of 13 male rats, aged 60 days, weighing from (180 to 200 g) were placed in special breeding cages in a clean room and controlled by the environment with a temperature of 20±2° C over a period of 24 hours, the mice received water and diet allocated to them for two weeks to adapt before starting the experiment. Rats were randomly distributed into 4 groups, where each group includes three rats, and each group represents one replicate. The groups were treated separately as follows: First group (control^{-}), they were fed normal rat food. The second group (control^{+}) was fed a high-fat diet while the third and fourth group were fed a high-fat diet and treated with 10 and 40 mg/body weight of β-glucan of β-glucan.

Rats Diet

Levin and Dunn-Meynell's formula, as stated in Levin & Dunn-Meynell (13), was used to modify the high-fat diet. The diet with a lot of fats is shown in Table (1), where all ingredients are from a well-mixed high-fat diet. Rats were fed the high-fat diet for 4 weeks to increase cholesterol levels.

Table 1. shows the normal and high-fat diets and their components.

The normal natural diet		The high-fat diet	
100%/gm	Nutrients	100%/gm	Nutrients
48.8	carbohydrates	43	carbohydrates
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	Ghee
13	Moisture content	20.0	Milk powder
8	ashes		
306.2	Total dietary energy (kcal/100 g)	414.0	Total dietary energy (kcal / 100 g)

Determination of serum lipid profile

The effect of the β-glucan compound with two different concentrations (10 and 40 mg/Bw) was studied on serum lipid profiles such as (cholesterol level, Triglycerides, LDL, HDL, and VLDL) and complete blood cells (CBC) such as (white blood cell WBC, Neurocytes, lymphocytes, monocytes, and Hemoglobin Hb).

Animal study design

After inducing obesity in rats for 4 weeks as described in (25), the weight and body mass index of the high-fat diet group were compared with the negative control group of rats. Random blood samples were taken, and

cholesterol levels, Triglycerides, LDL, and HDL were assessed. Daily oral doses of the beta-glucan compound at two concentrations (10-40) mg/body weight were given to the rats for a month.

Statistical Analysis

The complete randomized design (C.R.D) One-way ANOVA was used to analyze the data. The data were represented by the studied characteristics by means. Significant differences between the means were compared using the Least Significant Difference (L.S.D) test and the Duncan test at the level of $p < 0.01$. (1) Data were analyzed using the statistical analysis program GenStat v.12.1 (8).

Results and Discussion

The result of this current study revealed that β-glucan compound extracted from Lb. *fermentum* bacteria had a gummy texture, light creamy color, no taste, and is insoluble in water. β-glucan in a powder form, after drying in a hot air oven at 60º C for two days, had a dark brown color. The resulting compound was examined and confirmed with standard β-glucan using the FTIR test and photographic total sugar.

Effect of β-glucan on serum lipid profiles:

1.1 Effect of β-glucan on cholesterol level

The current results showed that β-glucan extracted from the bacteria *Lactobacillus fermentum* had a significant effect in reducing the rate of cholesterol $F_{(3, 8)} = 69.95$, $P<0.0001$). The results showed that β-glucan extracted from the bacteria *L. fermentum* at a concentration of 10 mg/Bw was superior in reducing the average cholesterol significantly to about (82.2mg/dl) compared to the average cholesterol (262 mg/dl) with the positive control comparison treatment ($Control^{+}$), whereas β-glucan at the concentration of 40 mg/Bw was less effective in lowering cholesterol level as the percentage of reduction was (163.9 mg/dl) compared to the positive control treatment as shown in the figure(1- A). This may be due to the concentration of 10 mg/Bw being the ideal concentration in reducing the level of cholesterol: the higher the concentration, the greater its reverse compound effect in reducing the cholesterol level.

1.2 Effect of β-glucan on triglycerides level

The current results showed that the β-glucan compound significantly reduced the level of triglycerides ($F_{(3,8)} = 36.21$, $P<0.0001$) at a concentration of 10 mg/Bw to about (29.54 mg/dl), compared to the level of triglycerides in the positive comparison treatment, which is about (162.33 mg/dl) as shown in Figure (1-B). While the concentration of 40 mg/Bw was ineffective in reducing lipids compared to the positive control treatment where the reduction rate was (61.9).

1.3 Effect of β-glucan on the level of low-density lipoprotein (LDL)

The β-glucan compound significantly reduced the rate of (LDL) ($F_{(3, 8)} = 31.11$, $P<0.0001$) at the concentration

of 10 mg/Bw to about (23.3 mg/dl) compared to the rate of LDL in the Control^{+}, which is about (155.53 mg/dl). On the other hand, the level of LDL was reduced to about (87.71 mg/dl) as shown in Figure (1-C) in treatment with a concentration of 40 mg/Bw β-glucan. These results are consistent with the findings of Naumann et al. (16) when studying the effect of β-glucan on blood lipid levels and lipoproteins where they found that β-glucan contributed to a reduction in LDL when combined with fruit syrup (16). Another study by MS Wolever (14) showed that taking a daily dose of 3g of β-glucan extracted from oats mixed with water for 4 weeks reduced the rate of LDL cholesterol in the blood compared to the comparison treatment that was a β-glucan free treatment where it was found that the average reduction of low-density lipoprotein (LDL) cholesterol was 6%. A study by Ho (10) revealed that using a concentration of β-glucan less than 3g/day was more effective in lowering LDL cholesterol than concentrations above 3 g/day when studying the effect of β-glucan in lowering LDL cholesterol. On the other hand, a study by Kerckhoffs et al., (11) found that bread and cakes rich in β-glucan did not affect the reduction of LDL cholesterol.

1.4 Effect of β-glucan on the level of very Low-density lipoprotein (VLDL)

The current results showed that the β-glucan compound extracted from the bacteria has a significant effect in reducing the level of VLDL ($F_{(3, 8)}$ = 6.227, P<0.001) at a concentration of 10 mg/Bw to about (5.91 mg/dl) compared to the rate of (32.47 mg/dl) in the control^{+} as shown in Figure (1-D). The β-glucan compound also showed a significant effect in reducing the level of VLDL at the concentration of 40 mg/Bw to about (12.38 mg/dl) compared to (32.47) in the control^{+}. A study conducted on animals using dogs showed a significant reduction in cholesterol, LDL and VLDL when using β-glucan at a high concentration of 10 g/kg of food (12). Another study also found the potential effects of oat-derived β-glucan in people at risk of metabolic syndrome, Data showed promising effects of glucan in lowering VLDL cholesterol levels and TG levels, but these effects varied based on the food used (3).

1.5 Effect of β-glucan on the level of high-density lipoprotein (HDL)

The current results showed that the β-glucan compound extracted from the bacteria has a non-significant effect in reducing the level of HDL ($F_{(3, 8)}$ = 4.61, P<0.037). Despite there being no significant effects among treatments, the β-glucan compound showed a rate of HDL of about (52.96 mg/dl) at the concentration of 10 mg/BW compared to the rate of (46.68 mg/dl) in the positive control+. The β-glucan compound also showed a high level of HDL at the concentration of 40 mg/dl to about (63.83 mg/dl) as it is shown in Figure(1-E). Rahar et al. (2011) demonstrated that β-glucan reduces LDL cholesterol and increases HDL, which may alleviate insulin resistance (18). Another study 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 (6). 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 (6). These changes occur due to adaptations in lipid metabolism, which increases lipoprotein enzymatic activity. Thanks to greater hydrolysis of TG-rich lipoproteins, thus causes less formation of hardened LDL and increases serum concentrations of nascent HDL (17).

Effect of β-glucan on complete blood cells CBC

2.1 Effect of β-glucan on White blood cells (WBCs)

The current results showed that the β-glucan compound had a non-significant effect on the rate of white blood cells WBCs ($F_{(3, 8)} = 1.62$, $P < 0.185$). in spite there were no significant differences in the number of WBCs among treatments, but the compound showed a slight reduction in the rate of the WBCs at the concentration of 10 mg/Bw to about ($7.43 \times 10^{*3}$ /µL) in comparison with the positive control ($Control^{+}$) as shown in Figure (1). It was found that β-glucan compound can enhance the participation of white blood cells in the immune response, which increases cell activity that improves phagocytic activity and cytokine secretion This 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 et al., on catfish fish by adding compounds containing at least 25% of β-1.3/1.6 glucans in feed the system can be improved through phagocytosis and increased phagocytic activity (27). 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 (2).

2.2 Effect of the β-glucan compound on Neutrophils (NEU)

β-glucan compound examined in this study showed a non-significant effect on the rate of Neutrophils ($F_{(3, 8)} = 4.71$, $P<0.035$). The results showed that there was a significant difference among treatments where the rate of (NEU) at a concentration of 10 mg/dl was about ($2.49 \times 10^{*3}$ /µL) compared to the positive control (Control+) which was about (0.78). In addition, there was no significant in the rate NEU at the concentration of 40 mg/Bw ($1.56 \times 10^{*3}$ /µL), compared to the positive control treatment ($0.78 \times 10^{*3}$ /µL) as in Figure (2).

2.3 Effect of the β-glucan compound on Lymphocytes

The results showed that the β-glucan compound has a significant effect on the rate of lymphocytes ($F_{(3, 8)} = 10.21$, $P<0.004$). Where it found that the rate of lymphocytes (Lym) increased at concentrations of 10 AND 40 mg/Bw ($9.73 \times 10^{*3}$/µL) and ($9.73 \times 10^{*3}$ /µL) respectively, compared to the positive control ($Control^{+}$) ($6.97 \times 10^{*3}$/µL) as it is shown in figure (1). The spleen, head, and kidneys are lymphatic organs connected to the immune system that are in charge of manufacturing

antibodies. They can be assessed indirectly by comparing organ weight to body weight, resulting in the somatic indicators of the spleen and kidneys (7). A study by Rohlenova showed marked differences in lymphocytes in fish during weeks 2 and 4 and about the rest of the weeks. The diet using β-glucan at a concentration of 0.05 in the second week showed higher values than other diets. This may be due to the proliferation of lymphocytes in the spleen. This organ, when it is stimulated immunologically, increases its activity and size, and vice versa, when it is immunocompromised, its size decreases. (21). Robertsen also stated that as much as increased intake of β-1.3/1.6 glucans do not mean that the immune response will improve in the same way for the organism, it must be taken into account that for each type must calculate the appropriate dose or concentration of β-1,3/1,6 glucans. This leads to an improved immune system. Otherwise, the initial battle against pathogens may be penetrated instead of boosting immunity (20).

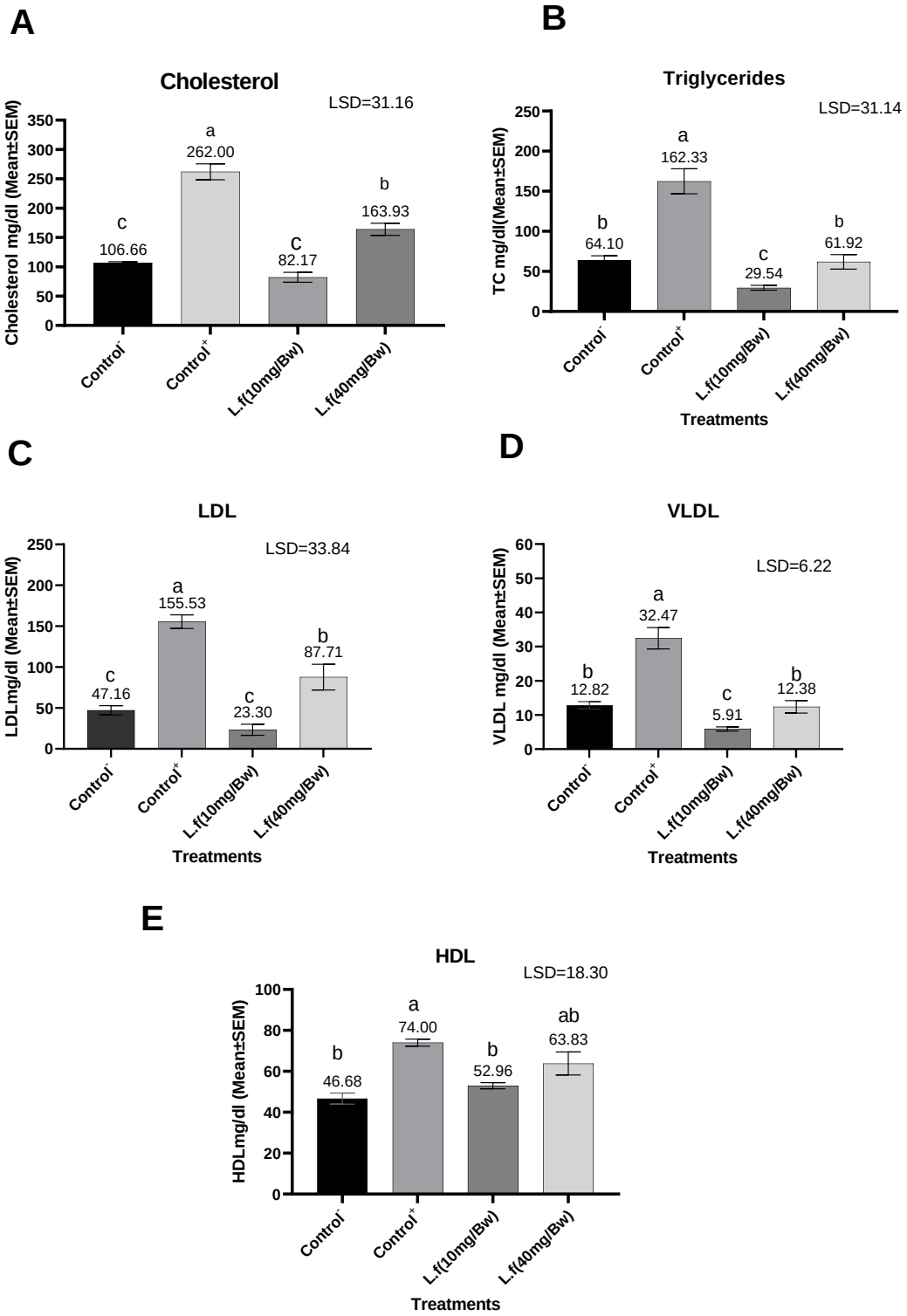

Figure 1. Bar charts representation of the effect of β-Glucan in Lipids profiles mg/dl (Mean±SEM). (A) Cholesterol level; (B) Triglycerides rate; (C) Low-density lipoprotein (LDL); (D) Very-low-density lipoprotein (VLDL) and (E) high-density lipoprotein (HDL).

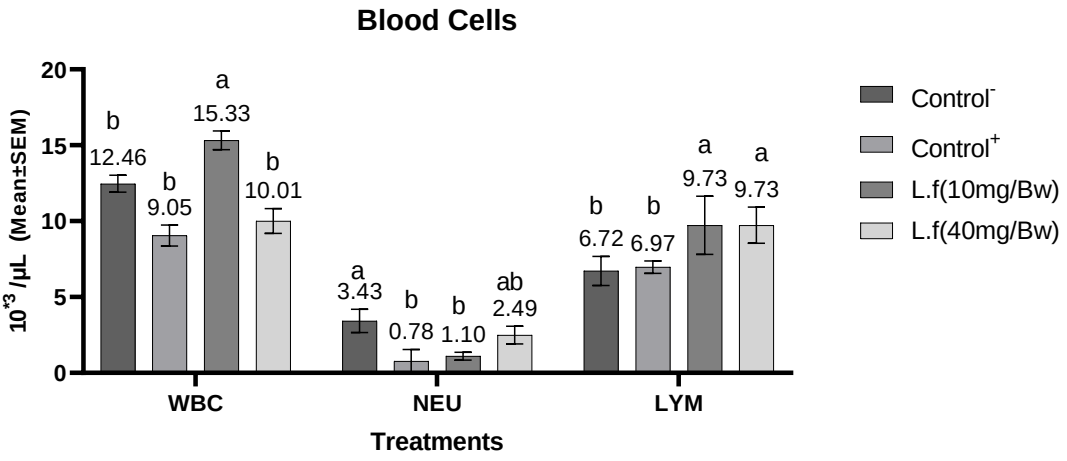

Figure 2. Bar charts representation the effect of β-Glucan compound on complete blood cells (CBC)10^{*3} /µL (Mean±SEM). WBC = White blood cell, NEU = Neutrophils and Lymphocytes.

Conclusion

β-glucan extracted from the bacteria *Lactobacillus fermentum* showed various effects on lipid profiles of the laboratory animals such as the level of cholesterol and the other lipids profils. In addition, β-glucan compound showed different effect on complete blood cells.

Conflict of interest

The authors have no conflict of interest.

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