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Extraction of Beta-Glucan from *Sacchromyces boulardii*, Study of its Chemical and Functional Properties, and Comparison of its Immunological Properties with Yeast Extracted from it

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Abstract

The study was conducted to identify the immunomodulatory effect of beta-glucan and to compare its immunomodulatory properties with *Sacchromyces boulardii* yeast. In this study, *S.boulardii* yeast was used to extract beta-glucan. The yeast was activated using a YPD medium, its purity was examined, and the following operations were performed on it: The biomass of *S.boulardii* yeast was grown in a vibrating incubator. The biomass was isolated, collected, washed, and dried. Extracting beta-glucan using the acid-base method for *S.boulardii* yeast is considered the best extraction method. Beta-glucan was partially purified using 4% phosphoric acid. Chemical tests were conducted on it and its functional properties (ability to bind to fats and water) were evaluated. The biological experiment was conducted for thirty days by taking three groups, each group containing five mice, A1 the control group, A2 the group fed with *S.boulardii* yeast, and A3 the group fed with beta-glucan extract. The effect of doses with treatments A1, A2, and A3 on the external appearance of the mice and the activity of the mice was studied. The effect of doses with treatments A2 and A3 was studied after the end of the starvation period on the final weight, and it was compared to treatment A1. It was noted that there were no significant differences at the level (P<0.05). Blood was drawn from mice by heart prick for the three groups, and immunological tests and antibodies IL 10, C3, and IgE were conducted. A significant increase was observed in the IL10 and C3 tests, while no increase in the IgE test.

Keyword: Beta glucan, biomass composition, *Sacchromuces boulardii*, IL10, complement factor 3, IgE .

1. Introduction

During the past decades, studies that explored the role of microorganisms that live in the human body have shown their great potential to treat and prevent diseases. Lactobacillus, Bifidobacterium, and *S. boulardii* species are the most commonly used, and they have been effective as a therapeutic option in treating several diseases, including gastrointestinal infections [1]. Probiotics are generally safe and well tolerated for conditions of the digestive system, and

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due to the imbalance in the intestinal microbiota, this led to the development of many diseases, including type 1 diabetes, cancer, and others, and using probiotics and Postbiotics, which has a beneficial effect on the host Ferment probiotics that have efficacy in inhibiting cancer cell lines with no effect on normal cells [2] . I knew yeast *S. boulardii* is a single-celled, sagittal feeding eukaryotic organism belonging to the genus Saccharomyces. It is possible to isolate *S. boulardii* from mangosteen fruits. The optimal temperature for the growth of *S. boulardii* is 37 ° C. Also, *S. boulardii* is more resistant to acidic extreme conditions, has a low pH, and stays longer in the gastrointestinal [3]. The most important characteristic of *S. boulardii* is that it can be adopted as a safe bioprosthetic for the treatment of many diseases, and the yeast boulardii is one of the yeasts that inhibit pathogenic [4]. The cell wall of *S. boulardii* is thicker than that of S. cerevisiae, which increases its defenses and resistance to survival towards extreme conditions, the use of *S. boulardii* in the preventive treatment of diarrhea associated with antibiotics in adults and also relapses that occur in patients with Clostridium difficile infection [5] (and used as nutritional supplements to prevent AIDS) [6]. and help treat stomach ulcers [7]. and beta-glucan is considered a prebiotic, as it is customary to extract beta-glucan from yeasts, and here the beta-glucan was extracted from the wall of *S.boulardii* yeast [8]. Beta-glucans also play a role as alarm molecules to activate the immune system through their binding to pattern recognition receptors (PRRs). Beta-glucans activate the inflammatory response. These receptors directly activate leukocytes to stimulate phagocytosis, cytotoxic and antimicrobial activity [9]. It also works to modify and enhance the production of cytokines that control the networks responsible for innate immune responses (and growth, improving survival, and beta-glucan has a prominent role in improving human resistance to bacteria and viruses such as infection with AIDS and others [10] . β-glucan has the ability to hold 9 times its weight in water and 1.4 times its weight in fat, and this enhances its role in food and some baked products [11].

2.Materials and methods

2.1 Biomass composition of *S. boulardii*

S. boulardi yeast is colonies with spherical, smooth, mucous, pale and white to creamy isolates [12]. The probiotic *S. boulardii* yeast was used in the form of lyophilized powder inside a capsule. The yeast was activated by emptying the content of one capsule of the lyophilized powder. yeast *S. boulardii* under sterile conditions in liquid YPD medium (Yeast extract, Pepton, Dextrose Broth) and incubated at a temperature of 37 C for 48 hours, (and the process was repeated three times under sterile conditions), the biomass was formed by preparing the prepared liquid PYD medium In a glass beaker, put 200 ml of the liquid medium, and put 7 ml of the third activation in each beaker. The beakers were placed in the shaking incubator at 200 revolutions per minute for four days, with proper ventilation of the beakers, and the temperature was 37 degrees Celsius. Using a centrifuge at 3000 cycles for five minutes, the biomass was separated. The precipitate was taken, washed three times with distilled water, and dried in a laboratory oven at a temperature of 60 degrees Celsius to the extent of dryness (for constant weight) [13].

2.2 Extraction of β-glucan from *S.bolardii* yeast

I took 200 dry yeast prepared in the previous paragraph and mixed it with 1 liter of 1.5 M of sodium hydroxide heated to (50-60) degrees Celsius, put the mixture in an autoclave for an hour, cooled the mixture to room temperature and left until the next day. Mix by centrifuge at 3000 rpm for 15 minutes. The filtrate was discarded, the precipitate was taken, and it was washed with

water three times with distilled water, using a centrifuge at 3000 rpm for 15 minutes. The precipitate was taken and mixed with a liter of glacial acetic acid at a concentration of 3%, then the mixture was heated to 85 degrees Celsius for three hours on a mixer. Then the mixture was cooled to room temperature and left for the next day. It was separated by centrifugation at 3000 rpm for 15 minutes. The filtrate was discarded and the precipitate was taken and mixed with a liter of distilled water. The mixture was separated by a centrifuge at 3000 rpm for 15 minutes. Discard the filtrate and take the precipitate and mix it with 600 ml of absolute ethyl alcohol. Heat the mixture with an electric stirrer until boiling, then leave it until the next day. Separate the mixture by centrifuge at 3000 rpm for 15 minutes. Discard the filtrate and take the precipitate and mixed it with 600 ml of acetone by electromixer. Separate the mixture by centrifuge at 3000 rpm for 15 minutes. Discard the filtrate, take the precipitate, mix with 600 ml of absolute ethyl alcohol, heat until boiling, and leave until the next day. Separate the mixture by centrifuge at 3000 rpm for 15 minutes. Discard the filtrate, take the precipitate, add a liter of distilled water to it, and heat Until boiling with good mixing and left until the next day and the upper layer was discarded and the washing was repeated with water three times with the use of a centrifuge each time and the filtrate was discarded. I kept the quantity produced until use. [14,15].

2.3 Purified β- glucan

The beta-glucan was treated with phosphoric acid 4%, at room temperature for two hours, the suspension was centrifuged and washed three times with distilled water to separate the leachate from the crude precipitate, then weighed and washed with distilled water, dried at a temperature of 37 °C, ground and stored until use [16]. Determination of beta-glucan concentration in the extract using High-Performance Liquid Chromatography (HPLC) technology.

The concentration and purity of beta-glucan were estimated [17]. Using the HPLC (High-Performance Liquid Chromatography) device, which was prepared by the Japanese company Shimadzu.

2.4 biological experiment

2.4.1. Experimental animals

Albino bulp-c white rats supplied by the Iraqi Center for Cancer Research and Medical Genetics / Al-Mustansiriya University were used, with a weight of approximately 183 gm, of male sex, and at the age of approximately 10 weeks. The three groups were fed a standard diet for three days, and water was always available at the animal needs , the first group A1 was left to feed on a standard diet throughout the experiment and returned as a negative control treatment, while the second group A2 was fed with a solution of *S.boulardii* The record, where the ratio of (5) mg of betaclocan per kg of body weight was chosen using a Teflon tube at an amount of (1) ml for each animal and after (30) days of dosing. Blood was drawn from the animals by the method of heart prick. Cardiac Puncture with a medical syringe. Then he studied the effect of nutrition on the rate of weight gain and blood tests, which include immunological tests.

2.4.2. Preparation of inocula

2.4.2.1 Preparation of group A2 dosage material

Regillier's milk was used, and due to the inability of *S. boulardii* yeast to metabolize milk sugar (lactose), table sugar (sucrose) was used to prepare the *S. boulardii* inoculum in high numbers, as the milk was prepared by sorting retrieved at a rate of 12% (w/v) and sucrose was added to it 1% (weight / volume) and autoclaved [18] then the milk was cooled to a temperature of 37°C and inoculated with 2% (volume/volume) of the liquid culture prepared from

S.boulardii yeast and incubated at a temperature of 37 °C. m until the appearance of coagulation, and the rats were dosed with (1) ml per day of the prepared product, for 30 days.
Dosage β- glucan (A3) The rats were dosed intragastrically with a preparation of β-glucan. [19]. The rats were weighed after (30) days of dosing, and the blood was drawn from the animals. Measurement of the level of the immune cytokine interleukin-10 (Interleukin-10).
For this purpose, the ELISA RAT IL-10 diagnostic kit was used to measure the levels of IL-10 in rat sera, according to the instructions of the American company EAGLE. The optical absorption readings were recorded from the ELISA reader. The standard curve (**Figure 1**) that represents the relationship Between absorbance and IL-10 concentration (pg/mL)

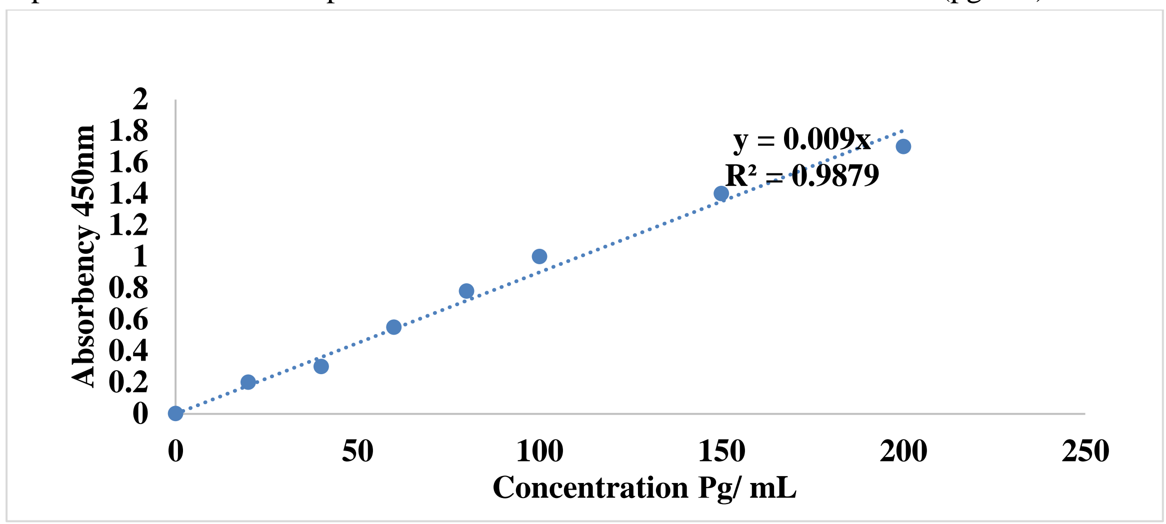

Figure 1. The standard curve of interleukin IL10.

2.4.3. Measurement of Complement factor 3 level and IgE concentration

For this purpose, the ELISA Complement Factor 3 diagnostic kit was used to measure Complement Factor 3 levels in rat sera, according to the instructions of the American producing company EAGLE. It was placed at room temperature and shaken before use to ensure homogeneity, and the steps attached to the instructions in the box were followed.

3.Results and Discussion

The results showed that the shape of the colonies was circular in color, white to creamy, and they were convex in shape with regular edges and a sticky creamy texture, with an average diameter of (1-2) mm when grown at a temperature of 37 ° C for 24 hours, and this agreed with [20, 21] .

We obtained 500 g biomass from dry yeast *S.boulardii* using the prepared PYD medium. This PYD medium was used because it is the best medium for yeast development, and pH (5.5) was the best in obtaining the largest biomass [22]. The ability of *S.boulardii* to resist extreme acidity is due to the presence of some proteins that are expressed in *S.boulardii* cells in the acidic environment resulting from metabolic effects given by the high physiological resistance to acidic conditions. Molecular mechanisms have been developed to respond to these conditions, which led to important developments in the representation of these cells and their cell membranes not being affected by extreme acidic conditions. *S. boulardii* was incubated at a temperature of 37 °C, as it has a shorter generation time at this temperature, as well as the ability to compete with organisms. Pathogenesis in the ecosystem of the digestive system, and this was agreed upon [23] The use of vibrating farms is attributed to the formation of biomass to obtain the highest possible

amount of it, the reason is that aeration and agitation are important things as they help to mix the contents of the medium and supply the growing cells with nutritional needs and oxygen to carry out metabolic activities [24].

3.1. Extraction of β-glucan from *S. boulardii* dry yeast

The base and acid method was used [25] because it saves time compared to other methods [26] and the weight of the obtained β-glucan was 7.370 g / 100 g of dry yeast. The yield was (36.8 g) of partially purified β-glucan, and this corresponds to [27] Chemical and physical properties and biological activity may vary according to the extraction method [28]. The base extraction process was carried out using the optimal extraction treatment, starting from the preserved yeast cell wall. At this stage, part of (protein +1-6 - β-glucan soluble in the base) is eliminated, then the mannoprotein precipitates, and the acid works to remove glycogen. In the last stage of extraction, disposal is done Partially purified β-glucan was obtained from fats using alcohol, and then a process of drying and grinding was carried out[29] showed that this method is characterized by its ability to extract β-glucan from yeast in a much higher quantity and more purification.

3.2. Purification of β-glucan

The weight of β-glucan after purification was (8.670 gm). By adding 4% phosphoric acid, the β-glucan extract was purified, and through this process, the filtered β-glucan (6-1) was separated from β-glucan (1-3). Crude precipitate. Some research indicates the ability of phosphoric acid to dissolve part of the mannoprotein as well [30].

3.3 Chemical checks

High-performance liquid chromatography for Glucan The results of the HPLC analysis shown in **Figures (2a)** and **(2b)** indicate that the standard β-glucan retention time corresponds to the β-glucan retention time under study, as the time required for both is (3.93 and 3.98) minutes. respectively, which confirms that the sample is an extract rich in beta-glucan, and the results also indicate a high concentration of β-glucan in the extract, indicating an effective method of extraction, and this is consistent with [31] and [32] that diagnosed β-glucan. Extracted from oat bran by HPLC technology, the retention time of the β-glucan standard model and the β-glucan model under study were identical, which was 3.137 minutes.

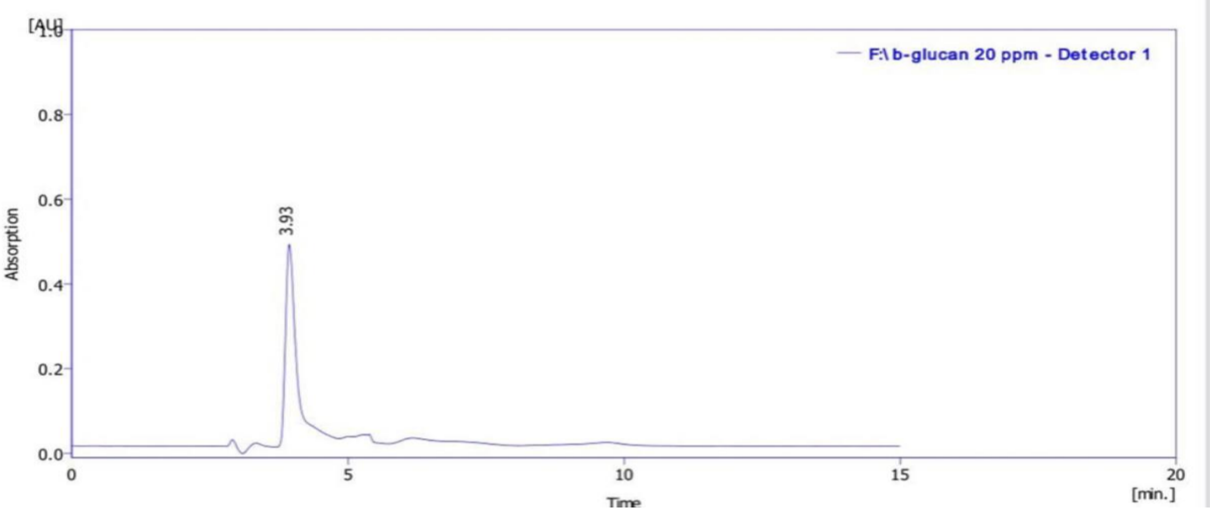

Figure 2a. HPLC of standard β-glucan of *S. boulardii* yeast

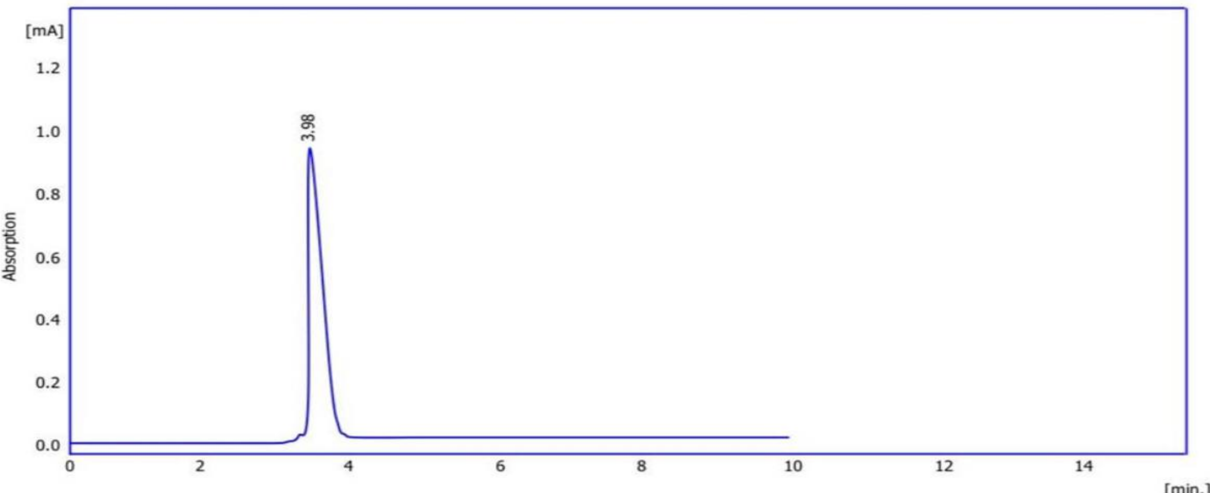

Figure 2b. HPLC of a sample of β-glucan extracted after purification from *S. boulasdii* yeast.

3.4. Effect of dosing with standard treatments and *S. boulardii* yeast - glucanβ on average weights of rats

Table 1. indicates the average weights of rats during the 30-day dosing period. It was noted that there was no difference in the effect of *S. boulardii* yeast and - β-glucan in the rate of weight gain that occurred between groups of rats, and it was noted that there were no significant differences at the level of (0.05) > P) in the average initial weights of rats among the studied treatments, which were 182.2 gm, 185.0 gm, and 183.0 gm for treatments A1, A2, and A3, respectively, while the average final weight was 223.2 gm (A1), 223.0 A2, and 219.0 gm A3, respectively. The mechanism of lowering cholesterol from *S.boulardii* results from its representation from its cells by the enzymes of the yeast cell wall [33] as it has the ability to reduce its level by 36.5%, The reason for this is due to the presence of fiber represented by betacocan in *S. boulardii* yeast, which gives a feeling of satiety and reduces the absorption of fat, which reduces the amount of food intake and delays the feeling of hunger, and then reduces the amount of energy supplied to the body, which results in a decrease in weight [34], and this is due to the presence of fibers that give a feeling of satiety and reduce the absorption of fat, which reduces the amount of food intake and delays the feeling of hunger and then reduces the amount of energy supplied to the body, and this results in a decrease in weight.

Table 1. The average initial and final weight, the difference in weights, and the rate of weight gain for rats treated with *S.bulardii* yeast and β-glucan, and compared with rats fed on the standard diet

Dosing type	Weight gain rate %	Average weight difference (g)	Average weight after the treatment (g)	The average weight before the treatment (g)
Control	22.46 a	41.0 a	223.2 a	182.2 a
S.boulardii	20 a	38 a	223.0 a	185.0 b
β-Glucan	19.64 a	36.0 a	219.0 a	183.0 b
LSD	**2.34**	**5.84^{gr}**	**5.044^{gr}**	**3.25^{gr}**

3.5. Measurement of the level of the immune cytokine interleukin-10 (Interleukin-10)

The results shown in **Figure 3** related to measuring the concentration level of the immune cytokine IL-10 by ELISA method showed that there was a statistically significant increase in the level of IL-10 concentration after doses of mice with treatment types (A2) and A3) compared with treatment The control (A1) at (P≤0.05*) level, and the results showed that there were

significant differences between the two treatments A2 and A3. The effect of β-glucan can be direct in stimulating immunity through its binding to the Dectin-1 receptor without the need for (TLR-2), which ultimately leads to enhancing the production of IL-10 [35] the results agreed with what was presented by [36], as it was shown that - glucanβ stimulates the process of expression (expression) of cytokines, which are proteins that have Characteristics similar to hormones that support the immune system of mammals, such as interleukin-1, interleukin-2, tumor necrosis factor, as well as interleukin 10 and 12.

The β-glucan, which is derived from the dead cell walls of *S. boulardii* yeast in the intestinal tract, has a strong effect in stimulating and increasing the immune response by binding to special receptors in the gastrointestinal tract, which stimulates immune cells to produce anti-inflammatory cytokines IL-10. This is explained by the fact that some Factors derived from the cell walls of the probiotic *S. boulardii* contributed to the enhancement and augmentation of the host immune response and the balancing of Th2/Th1 which promoted increased IL-10 production and that IL-10-producing B cells upregulated Th responses induced by 1,3-β-glucan [37].

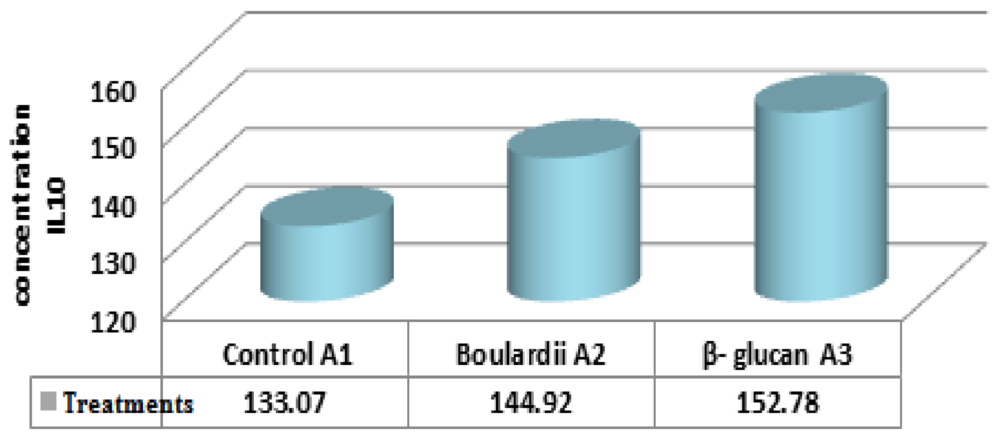

Figure 3. The effect of the three treatments on interleukin production

3.6. Measurement of the level of complement (C3) Complement factor 3

The results shown in **Figure 4.** related to measuring the level of C3 concentration by ELISA method showed that there was a statistically significant increase in the level of C3 after administering mice with the types of treatments (A2 and A3) compared with the control treatment (A1) at the level * ($P \leq 0.05$), The results also showed that there were significant differences between the two treatments A2 and A3, and the highest value was recorded for the A3 treatment of 1.69 ml/mg, followed by the A2 treatment with a concentration of 1.42 mg/ml, while the control treatment (A1) achieved a numerical value of 1.19ml/mg as the LSD was 0.337*.

The results agreed with [38] showing in light of his research that upon ingestion of β-glucans that are captured by macrophages via the Dectin-1 receptor and subsequently transported to the spleen, lymph nodes, and bone marrow, within the bone marrow, macrophages break the large proteins β-1,3-glucans into fragments. The smallest soluble is 3,1β-glucan. These fragments were subsequently taken up via complement receptor 3 (CR3) of granulocytes. These granulocytes containing β-glucan-fluorescein bound to CR3 were shown to kill inactivated

tumor cells after being ligated to the site of complement activation such as tumor cells coated with a monomeric antibody. Clonal and ample evidence has shown that β-glucan can act on several receptors, such as Dectin and complement receptor (CR3) [39].

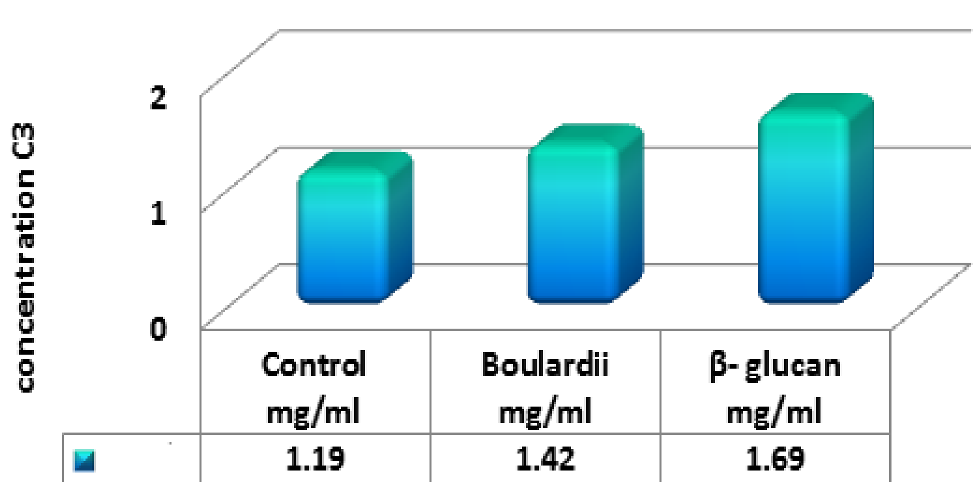

Figure 4. Shows the effect of the three treatments by stimulating the C3 sites

3.7. IgE screening

The results shown in **Figure 5** related to measuring IgE levels by ELISA method showed that there were no statistically significant differences in the level of IgE after administering mice with the types of treatments (A2 and A3) compared with the control treatment (A1). The results also showed that there were no significant differences. Between treatments A2 and A3, the highest value was recorded for treatment A3 was 120 ng/ml, followed by treatment A3 with a concentration of 118 ng/ml, while the control treatment (A1) achieved a numerical value of 116 ng/ml.These results are in agreement with [40] the IgE concentration should be low enough so that there are no adverse reactions attributable to the co-administration of beta-glucans in the clinical trial and given that oral beta-glucans were found to be less sensitizing than those given by injection and administration of *S. boulardii* in hydrolyzed formula extensively resulted in increased tolerance in infants with milk allergy compared to those treated with hydrolyzed formula alone, which was due in part to changes in the structure of the intestinal flora in the infants' gut and the result was no increase in IgE concentration [41].

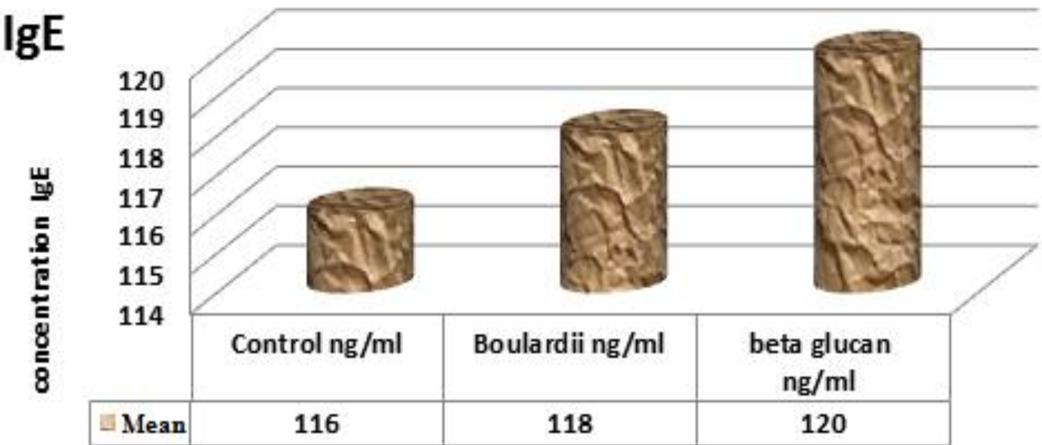

Figure 5. The effect of the three treatments on IgE secretion.

4.Conclusions

S.boulardii yeast was grown in a YPD medium, and it turned out to be the optimal medium for development. Beta-glucan was extracted from *S.boulardii* yeast using the acid-base method, which is considered the best method. This was demonstrated by HPLC testing. The biological experiment was conducted by feeding group A1 on The standard leech and group A2 on S.boulardii yeast solution and group A3 fed on beta-glucan extract showed, after conducting immunological tests, a significant increase to group A3 with tests (IL10, increased concentration of CO3 level and no increase in the concentration of IgE antibodies) and it turned out that beta-glucan It does not affect the increase in the body, but only increases the immune qualities of the body.

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Conflict of Interest

There are no conflicts of interest.

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References

1. Abdul-Hameed, R. F.; Al-Qadi, M. T.; Ibrahim, T. K.; Al-Khafaji, Z. M. Effect Of Different Conditions On Viability And Antagonistic Activity Of *Saccharomyces Boulardii* On Enteric Pathogenic Bacteria. *Baghdad Science Journal.* **2022**, *6, 4*. DOI: https://doi.org/10.21123/bsj.6.4.627-632.
2. Alziadi, R.; Alwaaili, J.R.; Allaibi, F.H. In Vitro Anti-Cancer Activity Of Sacchromyces Boulardii Metabolic Substances In Cancer Cells, *Int. J. Agricult. Stat.* **2020**, *16(1)*, 2079-2083.
3. Sheikh Zahir , Amer Abdel Rahman.; Al-Jilawi, Majed Hussain; Al-Ziyadi, Rahim Enad Khudair. Isolate *Saccharomyces Boulardii* From Mangsteen Fruits (Garcinia Mangostana L.), Molecular Identify And Study Its Ability To Reduce The Level Of Cholesterol And Inhibition of *E. coli* O157:H7 Bacteria, *Journal Of Al-Muthanna For Agricultural Sciences*. **2014**, *2, 2.*
4. Rezuqi, N.S.; Younis, F.Q. Inhibition Activity Of Saccharomyces Boulardii And Lactobacillus Acidophilus Against Pathogenic E. Coli Isolates From Recurrent Urinary Tract Infection In Women In Vitro, *Baghdad Science Journal.* **2014**, *11*, 1. DOI: https://doi.org/10.21123/bsj.11.1.22-32
5. Sarkar, S.R.; Mazumder, P.M.; Chatterjee, K.; Sarkar, A.; Adhikary, M.; Mukhopadhyay, K.; Banerjee. *Saccharomyces boulardii* ameliorates gut dysbiosis associated cognitive decline, *Physiology & Behavior*. **2021,** *236*, 113411. DOI: https://doi.org/10.1016/j.physbeh.2021.113411.
6. Ceccarelli, G.; Statzu, M.; Santinelli, L.; Pinacchio, C.; Bitossi, C.; Cavallari, E.N.; Vullo, V.; Scagnolari, C.; d'Ettorre, G. Challenges in the management of HIV infection: update on the role of probiotic supplementation as a possible complementary therapeutic strategy for cART treated people living with HIV/AIDS,*Expert opinion on biological therapy*. **2019**, *19(9),* 949-965. DOI: https://doi.org/10.1080/14712598.2019.1638907 .

7. Keikha, M.; Kamali, H. The impact of Saccharomyces boulardii adjuvant supplementation on alternation of gut microbiota after H. pylori eradication; a metagenomics analysis, *Gene Reports.* **2022**, *26*, 101499. DOI: https://doi.org/10.1016/j.genrep.2022.101499 .
8. Bashir, K.M.I.; Choi, J.S. Clinical and physiological perspectives of β-glucans: the past, present, and future, *International journal of molecular sciences*. **2017**, *18(9),* 1906. DOI: https://doi.org/10.3390/ijms18091906 .
9. Strandskog, G.; Sobhkhez, M.; Jørgensen, J.B.; Iliev, D.B. Composition of exosomes derived from Atlantic salmon (*Salmo salar*) head kidney leukocytes. **2015.** DOI: https://doi.org/10.3389/fimmu.2021.736964 .
10. Hoenigl, M.; Lin, J.; Finkelman, M.; Zhang, Y.; Karris, M.Y.; Letendre, S.L.; Ellis, R.J.; Burke, L.; Richard, B.; Gaufin, T.; Isnard, S. Glucan rich nutrition does not increase gut translocation of beta-glucan, *Mycoses.* **2021,** *64*(1), 24-29. DOI: https://doi.org/10.1111/myc.13161.
11. Al-Aithy, S.A.; Abdalrahman, A.; Musa, M.A. The Antimicrobial Activity Of Barley Beta Glucan And Its Use As Prebiotic In Acidophilus Milk, *Iraqi Journal of Agricultural Sciences* **2012**, *43(2)*, 109-118. DOI: https://doi.org/10.58629/ijaq.v19i2.419 .
12. Pais, P.; Almeida, V.; Yılmaz, M.; Teixeira, M.C. *Saccharomyces boulardii*: what makes it tick as successful probiotic? *Journal of Fungi.* **2020**, *6(2)*, 78. https://doi.org/10.3390/jof6020078 .
13. Mohammed, I.Y.; Abakr, Y.A.; Kazi, F.K.; Yusup, S.; Alshareef, I. ; Chin, S.A. Comprehensive characterization of Napier grass as a feedstock for thermochemical conversion, *Energies*. **2015**, *8(5)*, 3403-3417. DOI: https://doi.org/10.3390/en8053403 .
14. Donzis, B.A.; Donzis Byron A. Method for revitalizing skin by applying topically water insoluble glucan,*U.S. Patent*. **1993**, 5(223),49. DOI: https://doi.org/10.1016/0010-4361(93)90264-9.
15. Al-Shouki, R.M.M.; Nasser, A.K. Extracting β-glucan from *Saccharomyces cerevisiae* and using it as an alternative to fat to improve certain qualities in refrigerated beef, *Journal of Al-Muthanna for Agricultural Sciences*. **2019**, *7(3)*. DOI: https://doi.org/10.33762/bjas.2019.161200.
16. Petravić-Tominac, V.; Zechner-Krpan, V.; Berković, K.; Galović, P.; Herceg, Z.; Srečec, S. ; Špoljarić, I. Rheological properties, water-holding and oil-binding capacities of particulate β-glucans isolated from spent Brewer's yeast by three different procedures, *Food technology and biotechnology*. **2011**, *49(1),* 56-64. DOI: https://doi.org/10.1556/aalim.40.2011.2.12 .
17. Alaubydi, M.A. ; Abed, R.M. The Usage of ß-glucan Extracted from Local Mushroom as Immunomodulator, *Current Research Journal of Biological Sciences*. **2011**, *3*(*5*), 535-541.
18. Baron, E.J.; Peterson, L.R. ; Finegold, S.M. Mycobacteria, *Baily and Scott's Diagnostic Microbiology. 9th ed. St. Luis: The CV Mosby Company*. **1994**, 590-633. DOI: https://doi.org/10.1099/00222615-42-4-308.
19. Pengkumsri, N.; Sivamaruthi, B.S.; Sirilun, S.; Peerajan, S.; Kesika, P.; Chaiyasut, K. ; Chaiyasut, C. Extraction of β-glucan from *Saccharomyces cerevisiae*: Comparison of different extraction methods and in vivo assessment of immunomodulatory effect in mice. *Food Science and Technology*. **2016**, *37*, 124-130. DOI: https://doi.org/10.1590/1678-457x.10716 .
20. AL-Zaiadi, R.; AL-Shekdhaher, A. Isolation and Identification of food Probiotic *Saccharomyces boulardii* by using traditional methods, Vitek 2 system and molecular identification methods, *iraq journal of market research and consumer protection*. **2016**, *8*. DOI: https://doi.org/10.9734/bbj/2016/22927.
21. Al-Jilawi, M.H.; Al-Sheikh D.A.A.; Al-Ziyadi, R.I.K. Physiological Properties From Probiotic Saccharomyces boulardii that isolates from Mangosteen fruits and diagnosed molecularly, *Euphrates Journal of Agriculture Science*. **2015**, *7,* 1.
22. Karthik, A.; Sudalaimani, K.; Vijayakumar, C.T.; Saravanakumar, S.S. Effect of bio-additives on physico-chemical properties of fly ash-ground granulated blast furnace slag based self-cured geopolymer mortars, *Journal of hazardous materials*. **2019**, *361*, 56-63. DOI: https://doi.org/10.1016/j.jhazmat.2018.08.078 .

23. McFarland, L.V. Common organisms and probiotics: *Saccharomyces boulardii*, In*: The microbiota in Gastrointestinal Pathophysiology*. **2017**, 145-164. DOI: https://doi.org/10.1016/b978-0-12-804024-9.00018-5 .
24. Badr, H.; El-Baz, A.; Mohamed, I.; Shetaia, Y.; El-Sayed, A.S.; Sorour, N. Bioprocess optimization of glutathione production by *Saccharomyces boulardii*: biochemical characterization of glutathione peroxidase, *Archives of Microbiology*. **2021**, *203*, 6183-6196. DOI: https://doi.org/10.1007/s00203-021-02584-0 .
25. Donzis, B.A.; Donzis Byron A. Method for revitalizing skin by applying topically water insoluble glucan, *U.S. Patent*. **1993**, *5(223),* 491.
26. Al-Jumaiee, S.A.; Al-Manhel, A.J.; Al-Hussainy, K.S. The Effect of Adding Beta-glucan Extracted From Bread yeast and barley bran on Inhibiting Microbial Population of Fish Patties at Cooling Storage Periods. *Iraqi Journal of Aquaculture* **2021**, *18(2),* 47-62. DOI: https://doi.org/10.58629/ijaq.v18i2.350.
27. Al-Rubaee, A. Extraction of glucan from yeast Saccharomyces cerevisiae. M.Sc. Thesis, *College of Science – Baghdad University*. **2008.**
28. Courie, P.; Patelakis, S.; Miles, A. Progressive BioActives Inc. A Method of Producing an Economical and Ecologically Sound Natural Immunobiotic Extract for Use as a Health Management Instrument and a Replacement for Growth Promotion Antibiotics in Livestock and Companion Animals, *U.S. Patent Application* . **2005**, *10(711),* 980.
29. Huang, M.H. ; Yang, M.C. Evaluation of glucan/poly (vinyl alcohol) blend wound dressing using rat models, *International journal of pharmaceutics*. **2008**, *346(1-2),* 38-46. DOI: https://doi.org/10.1016/j.ijpharm.2007.06.021.
30. Petravić-Tominac, V.; Zechner-Krpan, V.; Berković, K.; Galović, P.; Herceg, Z.; Srečec S, Špoljarić, I. Rheological properties water-holding oil-binding capacities of particulate β-glucans isolated from spent Brewer's yeast by three different procedures, *Food technology and biotechnology*. **2011,** *49(1),* 56-64 . DOI: https://doi.org/10.1016/j.jbiotec.2017.06.052.
31. Ibrahim, H.K.; Mahdi, M.S. Antioxidant Effect of Beta-glucan Extract from *Saccharomyces Cerevisiae, Indian Journal of Forensic Medicine & Toxicology*. **2020**, *14(4),* 2066-2072. DOI: https://doi.org/10.37506/ijfmt.v14i4.11853.
32. Al-Azzawi, Shaima Saadi Lafta. The use of beta-glucan and inulin to improve the physicochemical, rheological and nutritional properties of low-fat mozzarella cheese. *PhD thesis-College of Agriculture - University of Baghdad*. **2018**. DOI: https://doi.org/10.36295/asro.2020.231622 .
33. Saady, S. Study Effect of Feeding on Low Fat Mozzarella Cheese Using Fat Mimetic such as Beta–Glucan and Inulin in some Health and Physiological Indicators of White Mice, *Tikrit Journal for Agricultural Sciences*. **2017**, *17*, 813-826.
34. Al-Shammary, H.A.A.; Daher, A.A.A.S. ; Salih, S.M. Effect of probiotic *Saccharomyces boulardii* in weight losing and cytokines levels in mice before and after Infection by Salmonella typhimurium, *Al-Anbar Journal of Veterinary Sciences*. **2018**, *11*, 1. DOI: https://doi.org/10.37940/ajvs.2018.11.1.8.
35. Basso, A.M.M.; De Castro, R.J.A.; De Castro, T.B.; Guimarães, H.I.; Polez, V.L.P.; Carbonero, E.R.; Pomin, V.H.; Hoffmann, C.; Grossi-de-Sa, M.F.; Tavares, A.H.; Bocca, A.L. Immunomodulatory activity of β-glucan-containing exopolysaccharides from Auricularia auricular in phagocytes and mice infected with Cryptococcus neoformans. *Medical mycology*. **2020**, *58(2),* 227-239. DOI: https://doi.org/10.1093/mmy/myz042.
36. Pelizon, A.C.; Kaneno, R.; Soares, A.M.V.C.; Meira, D.A. ; Sartori, A. Immunomodulatory activities associated with β-glucan derived from Saccharomyces cerevisiae, *Physiological research*. **2005,** 557-564. DOI: https://doi.org/10.33549/physiolres.930686.

37. Zhang, Q.; Lu, Y.; Liu, F. IL-10 producing B cells regulated 1, 3-β-glucan induced Th responses in coordinated with Treg. *Immunology Letters*. **2021**; *235*, 15-21. DOI: https://doi.org/10.1016/j.imlet.2021.04.011 .
38. Chan, G.C.F.; Chan, W.K. ; Sze, D.M.Y. The effects of β-glucan on human immune and cancer cells. *Journal of Hematology and Oncology*. **2009**, *2(1),* 1-11. DOI: https://doi.org/10.1186/1756-8722-2-25.
39. Baert, K.; Sonck, E.; Goddeeris, B. M.; Devriendt, B.; Cox, E. Cell type-specific differences in β-glucan recognition and signalling in porcine innate immune cells, *Developmental and Comparative Immunology*. **2015**, *48(1),* 192-203. DOI: https://doi.org/10.1016/j.dci.2014.10.005.
40. Jesenak, M.; Hrubisko, M.; Majtan, J.; Rennerova, Z.; Banovcin, P. Anti-allergic effect of pleuran (β-glucan from *Pleurotus ostreatus*) in children with recurrent respiratory tract infections, *Phytotherapy Research*. **2014**, *28(3),* 471-474. DOI: https://doi.org/10.1002/ptr.5020.
41. Plaza-Diaz, J.; Ruiz-Ojeda, F. J.; Gil-Campos, M; Gil, A. Mechanisms of action of probiotics. *Advances in Nutrition*. **2019**, *10,(l-1),* 49-S66. DOI: https://doi.org/10.1093/advances/nmy063 .