Extraction and characterization of the beta-glucan from *Saccharomyces boulardii* with detecting its effect on the immune response

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Abstract

The aim of this research was to study the immunological properties of beta-glucan extracted from *S.boulardii* yeast. after activating the *S.boulardii* .The biomass was formed using the optimal culture medium, and the beta-glucan was extracted using the base and acid method, and the chemical and physical properties were studied.The immunological properties were studied after conducting the biological experiment on two groups of rats, group A1, which is the control group, and group A2, which is the group that was fed beta-glucan at a dose of (5) mg.kg-1 of weight and they were dosed with (1) ml of beta-glucan solution, and after conducting the biological experiment for 30 days, and immunological examinations were performed for the two groups.The following immunological tests were coundected: IL10, IgM and IgE tests, and the results were compared between the two groups. Significant differences were observed at the level of P<0.5 for IL and IgM tests, and there was no significant difference in IgE tests, and it was noted that there was no weight gain for the rats after the dosing period. Where the results proved that the beta-glucan extract is one of the immune compounds that showed its efficiency in prevention and treatment.

Key words: b-glucan; anticarcinogenic activity; monoclonal antibodies; IL10,b-glucan

Introduction

Beta-glucans are a group of the most abundant forms of polysaccharides with significantly different physicochemical properties dependingt on the source. It has been well studied as . Structurally, Beta-glucans comprised entirely of glucose monomer that arranged linearly and bound by glycosidic bonds at β-1,3 and β-1,4 or β-1,6.

with glucose polymers connected by a linear β-glycosidic 1 → 3 chain axis. Branching into β-glycosidic chains 1 → 4 or 1 → 6, their physical and [1][2].Beta-glucan is a well-researched. β-glucans are a group of glucose polymers.Much is known about the molecular mechanisms of β-glucans and their binding to specific cellular receptors (for example, Dectin-1) along the lining of the gut and to specific immune cells, such as antigen-presenting cells (APCs) [3,4] Beta-glucan is one form of soluble dietary fiber. Several studies have shown that beta-glucan possesses immunomodulatory properties, depending on its structure, solubility, and molecular weight [5].

β-glucans particles derived from fungi including yeast, which consist of a (1,3)-linked backbone with small numbers of (1,6) β-linked side chains, are known to stimulate immunomodulation [6,7]. Besides the observed immunomodulatory effects, there is increasing evidence that beta-glucans may have prebiotic and Thus it may modify microorganisms [8]. Traditionally, beta-glucans were extracted from yeasts, especially baker's yeast *S.cervisiae* . In the present study, however, beta-glucans were extracted from the wall of *Saccharomyces. boulardii* yeast. Boulardii yeast is one of the yeasts that inhibit pathogenic bacteria [9]. The total cell wall thickness of *S. boulardii* is greater than that of the *S. cerevisiae*. The differences in cell wall thickness and composition of *S. boulardii* may explain some unique microbiota characteristics found in this species. Previous studies have found that certain cell wall components, including β-glucans, increase the resistance of probiotic bacteria to pH stresses

and GI conditions. It has been proved the significant role of the yeast cell wall in the resisting external pressures [10]. Moreover, *S. boulardii* is [11]. Additionally, beta-glucans have been applied in the food industry depending on its physicochemical properties, . Beta-glucan and its derivatives have medical and pharmacological effects. Also, beta-glucans extracted from yeasts and fungi are distinguished by their excelled on other species as immune response modulators, in addition to their immunomodulatory effects and their anti-cancer agent]. In recent years, a clear association between nutrition and cancer has been highlighted by several epidemiological and research studies,[12].Indeed, despite their structural function, some β-glucans exert important potential in biological activities. [13,14]. Moreover, β-glucan can modulate the immune response of the host by activating innate immune cells including macrophages, neutrophils, and granulocytes,[15].

Materials and methods:

Yeast source and cultur preparation

The probiotic yeast *Sacchromyces boulardii* was used in this study and prepared by the Department of Food Sciences, College of Agriculture /, University of Baghdad, Iraq in the form of lyophilized powder inside a capsule. The yeast was activated by emptying the content of one capsule of *S.boulardii* under sterile conditions in the liquid Yeast extract, Peptone, Dextrose Broth medium (YPD). The medium was prepared by dissolving 1% yeast extract, 2% peptone, and 2% dextrose in distilled water for the purpose of activation and growth of *S. boulardii* isolate [16]. The culture was incubated at 37 oC for 48 hours, and the process was repeated in triplicates .

Extraction of β-glucan from S.bolardii

The prepared PYD liquid medium was distributed 200 ml in each flask (500 ml) and 7 ml of the third activation was placed in each flask. The flasks were placed in shocking incubator at 200 rpm at 37 °C for 4 days. The cell biomass was obtained after centrifugation at 3000 rpm for 5 min. The precipitate was taken, washed three times with distilled water, and dried at 60 °C in a laboratory oven [17]. The BYRON method was used to extract the beta-glucan, and the extract was dried in the oven with a hot air stream at 40 °C. The produced quantity was preserved until use. [18,19].

Purified β -glucan

Treatment with phosphoric acid 4% of the extract obtained from the extraction process in the above paragraph, at room temperature for two hours. Then, the suspension was centrifuged and washed three times with distilled water to separate the leachate from the crude precipitate. The leachate was dried at a temperature of 37 °C, ground and stored until use [20].

Physical and chemical properties of the purified β-glucan

The extracted beta-glucan was analyzed using High-Performance Liquid Chromatography (HPLC).

This technique was used to separate, identify, and quantify the concentration of beta-glucan. the Japanese company Shimadzu [21].

Fourier transform infrared spectrometer (FTIR)

The chemical composition of β -glucan of *S. boulardii* was analyzed by Fourier transform infrared spectrometer (Shimadzu IRAffinity - Japan) at Department of Environment and Water, Ministry of Science and Technology in Iraq.

Biological experiment

Experimental animals and their weights

Albino balb-c white mice were used and supplied by the Iraqi Center for Cancer

Research and Medical Genetics, Al-Mustansiriya University. The mice were …10 weeks old male weighting about 183 gm. The experiments were divided into two groups: experimental group was 10 rats dosed on a solution of A β-glucan under study and a group of control rats fed on the standard diet and studying the effect of feeding on the rate of weight gain and blood tests that include immunological tests and the number of white blood cells. The rats were weighed before and after the dose. Later, the weights of internal organs of mice such as liver, spleen, and kidneys were measured after being applied the doses. The animals were placed in controlled conditions in terms of ventilation and temperatures ranging 25 + 2 ° C under 12 hours of uninterrupted dark each day. At the beginning of the experiment, the two groups were fed a standard diet for three days, and water was always available. The first group considered a control treatment, A1, was left to feed on a standard diet throughout the duration of the experiment. The second group, A2, was treated The ELISA RAT IL-10 diagnostic kit was used to measure the IL-10 levels in rat sera according to the instructions of the American company EAGLE Drawing the standard curve with a solution of β-glucan at the rate of (1) ml of the prepared solution, where the ratio of 5 mg . kg-1 of body weight was chosen using a Teflon tube with an amount of 1 ml for each animal. The rats were weighed after 30 days of treatments. Blood was drawn from the animals by cardiac puncture by means of a sterile medical syringe. The rats were sacrificed to remove the liver, spleen, and intestines, and the internal organs: liver, spleen, and kidneys were weighed. The organs were washed well with running water and then preserved in a 10% formalin solution [22].

Immunological tests

Immunological tests were conducted at the University of Technology and in private laboratories which included the below tests:

Measurement of the level of the immune cytokine interleukin-10 (IL-10)

(Fig. 1), which represents the relationship between the absorbance and the concentration of interleukin-10 (pg/ml).

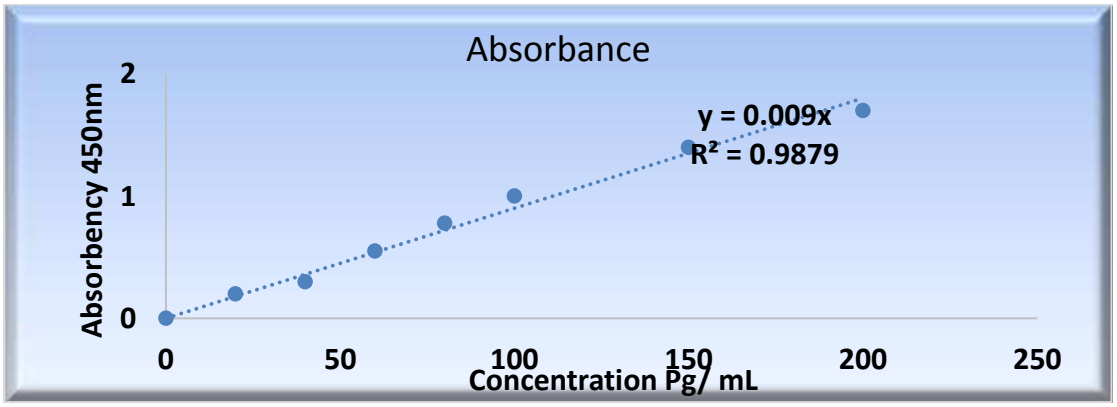


Figure (1) Standard curve for interleukin IL10

Measurement of the level of IgE, IgM ELISA ASSAY

The diagnostic kit RAT, IgE, IgM ELISA ASSAY KIT (company name and country) was used to measure separately the levels of IgE and IgM in the rat serums according to the company instructions.

Statistical analysis

The statistical program System Analysis Statistical-SAS (2012) was used to analyze the presented results. To study the effect of different treatments on the traits under study, the significant differences between the averages were compared with the Least Significant Difference (LSD) test at the level of probability. $P < 0.05$.

Results and discussion

***S. boulardii* activation and biomass formation**

The activation process was conducted for three times to ensure obtaining a starter with the maximum live numbers, where it was found that repetition in the activation process is necessary in activating new metabolic pathways depending on the type of sugar in the medium, whose regulation is linked to the relevant genetic genes, 500 g dry yeast was harvested from *S.boulardii* biomass culture flasks. The prepared YPD medium was used, and this was agreed upon in the studies, as it was found that the best growth of *S.boulardii* yeast was found on YPD medium [19].A solution (0.1) of hydrochloric acid was used for the purpose of converting the medium used for activation to acidic at a pH of (5.5). It was found that the best biomass was obtained through a medium whose pH was (5.5) Because it grows in an acid medium. . [23]. The ability to resist low pH is one of the main criteria for selecting probiotic strains to be able to reach the small intestine and withstand the extreme pH in the stomach.[24]It was found that some of the proteins that are expressed in *S. boulardii* cells in the acidic environment are caused by metabolic effects that give them high physiological resistance to acidic conditions., and also that *S. boulardii* cells developed molecular mechanisms to respond to these conditions. It led to important developments in the representation of these cells and the non-affection of their cell membranes [25]. The strains of *S. boulardii* were able to grow faster at 37 °C compared to *S. cerevisiae* which gives *S. boulardii* an important advantage for using as a probiotic to compete with pathogenic organisms in the human digestive system (reference) The use of the rocking incubator for a period of 96 hours gave the best results in terms of cell growth and biomass formation, and when the time period increased, it led to a decrease in the biomass, where it leads to a decrease in yeast productivity due to self-decomposition and nutrient depletion from the medium [26]. Because *S. boulardii* yeast does not possess the enzyme -galactosidase β, which is responsible for the metabolism of lactose, attention has been directed to the use of sugar substitutes as a source of carbon that can fulfill the required purpose in the growth of yeast cells [27]. Therefore, sucrose was adopted due to its availability and the ability of yeast to metabolize this sugar, which is due to its possession of the enzyme invertase within its enzymatic system.

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

β-glucan was extracted from the cell wall of dry S. boulardii using the acid-base method. This method was chosen due to its ability to extract beta-glucans from yeast in a much higher quantity, more purification [28], limited use of organic solvents, and time savings compared to other beta-glucan extraction methods. The results showed that the yield of beta-glucan extracted from S. boulardii was more than that extracted from yeast *S.cerecisiae* .The extraction process was carried out in a base, which is the optimal treatment, starting from the

preserved yeast cell wall. At this point, part of the protein +1-6-soluble beta-glucan is eliminated into the base. Then the mannoprotein precipitates, and the acid works to remove the glycogen. In the last stage of extraction, the lipids are degreased with alcohol, so we obtained partially purified beta-glucan, after which drying and grinding took place [29]. In this way, the weight of the resulting beta-glucan was 7.370 g/100 g dry yeast of dry yeast. The yield was 36.8 g of partially purified beta-glucan. Research indicated that this method was able to obtain 6.25 g/100 g glucan from S. boulardii yeast. Dry weight β-glucan can be extracted from yeast to varying degrees of purity depending on the method used; The chemical and physical properties and biological activity may vary according to the extraction method [31,30] and research showed that the yield of β-glucans from yeasts using the acid base was approximately as high as 12% [31].

Purification of β-glucan

The β-glucan extract was purified by adding phosphoric acid, where through this process the separation of - glucan - β (6-1) from β glucan - 3 -1and phosphoric acid has the ability to dissolve part of the mannoprotein, Phosphoric acid was added at a concentration of 4% to the extract obtained from the extraction process to separate the leachate (containing β-(6 -1)β-glucan from the precipitate that represents crude β-(3-1)β-glucan [32]. Good methods for separating the components of the yeast wall, especially β-glucan, and the reason for the difference in the results may be due to the differences in the genetic material of the strains yeasts and the method of extraction [33].

Study of the chemical and physical properties of purified β-glucan

The results of the HPLC analysis showed that the retention time of the extracted β-glucan had the same retention time of the standard β-glucan (Fig. 1a). One peak was appeared 3.98 min. of the liquid samples of the β-glucan extracted form S. boulardii (Fig. 1b). Similarly, the result revealed one peak 3.93 min. of the liquid samples of the standard β-glucan (Fig. 1a). These results confirmed the purity of the extracted β-glucan Additionally, the results indicate a high concentration of β-glucan in the extract, indicating an effective method of extraction AND agreed with [34]

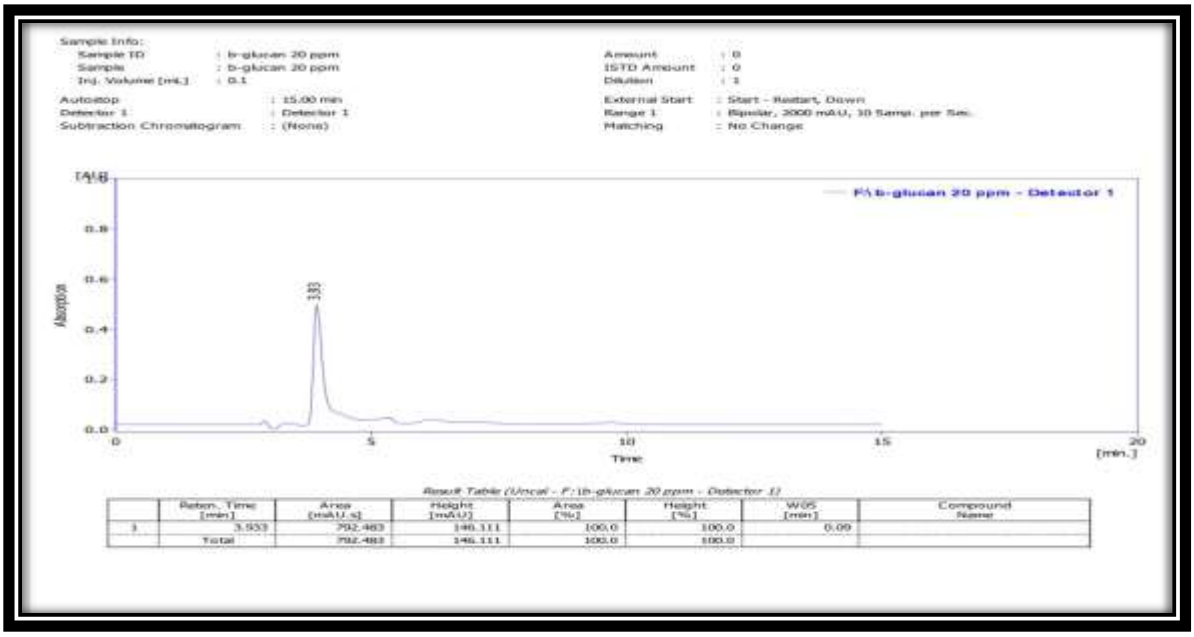


Figure (1a) HPLC of a sample of standard β-glucan

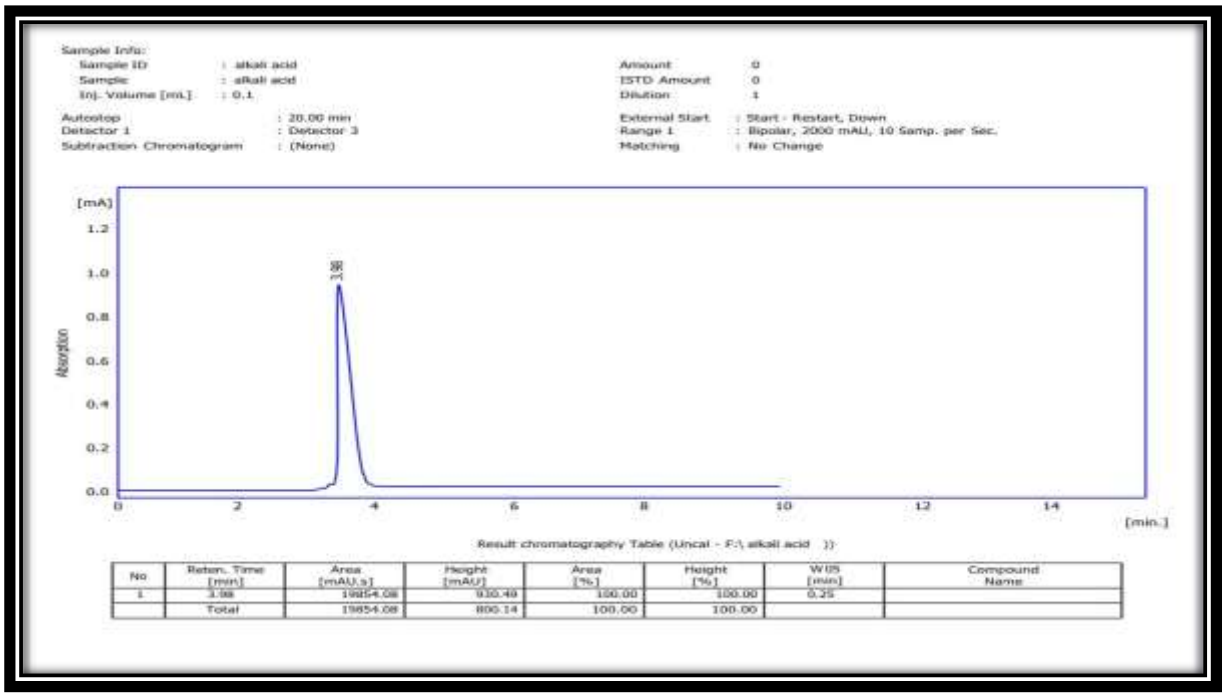
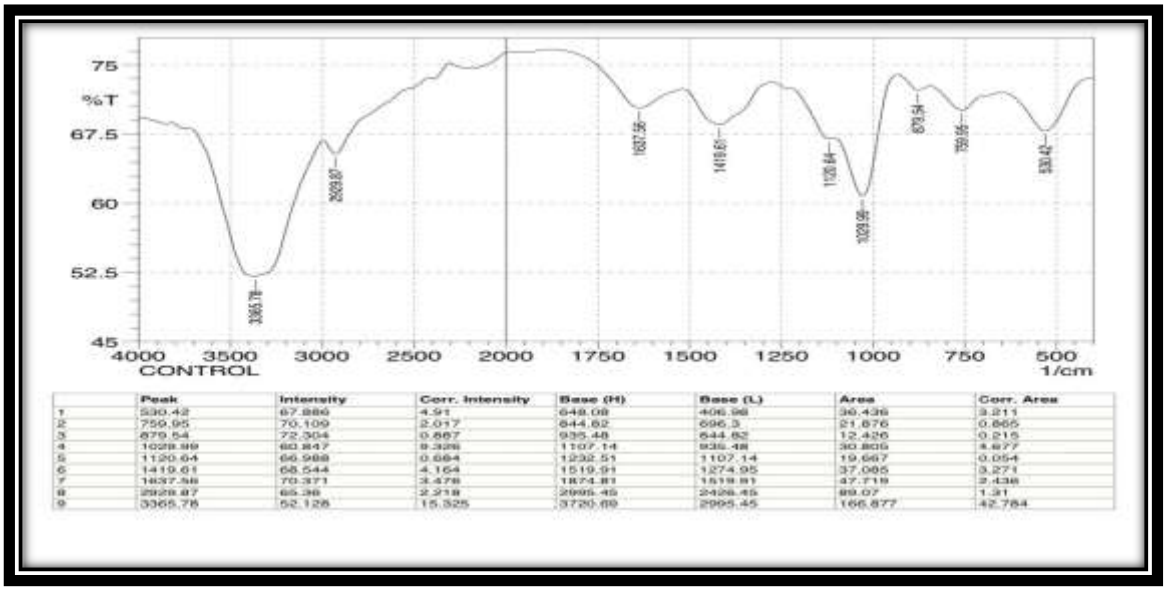


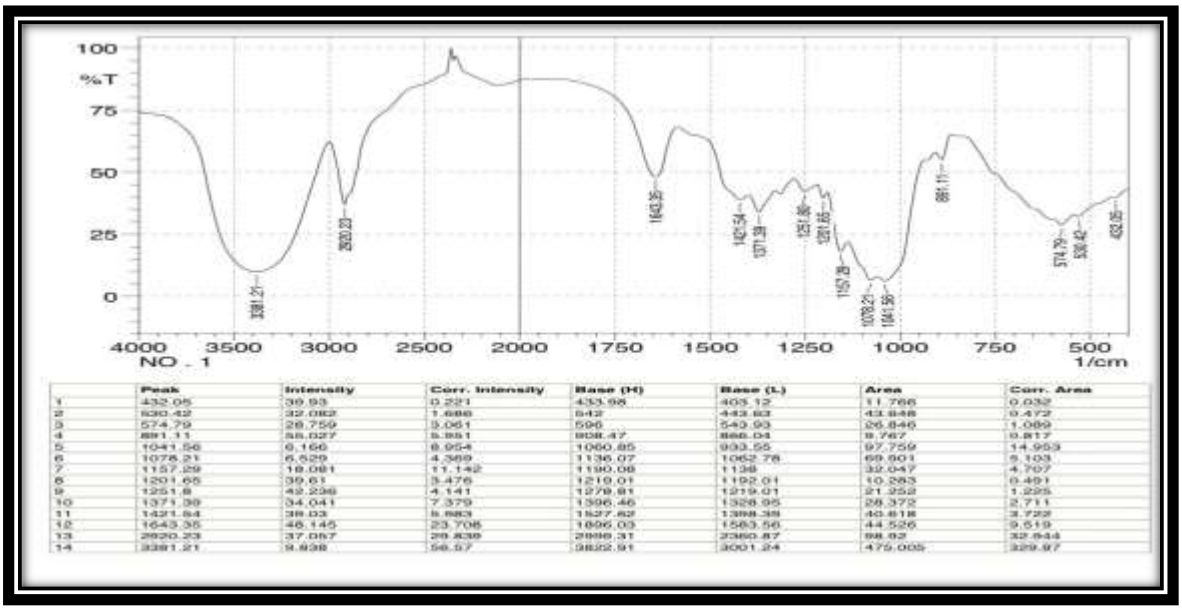
Figure (1b) HPLC of a sample of β-glucan of the extract

Fourier transform infrared spectrometry (FTIR)

The β-glucan was analyzed using FT-IR spectroscopy to detect the functional group in the β-glucan chemical structure and compared these groups with the standard groups. FT-IR technology is used to characterize organic molecules by detecting active groups and boundaries present in the molecule [40].The result in Figure (2a) showed that the infrared spectrum at absorption 1041.56 cm-1 means the presence of C-O bonds, which is a distinctive feature of the extended β-glucan structure with the standard 1029.99 cm-1 (Fig. 2b) AND agreed with [35]. The absorbance at 1421.54 cm-1 indicates the presence of C–H aliphatic curvature; The standard absorption was at 1419.61 cm -1 AND agreed with [36,37,38,]



	Peak	Intensity	Corr. Intensity	Base (H)	Base (L)	Area	Corr. Area
1	530.42	67.886	4.91	648.08	496.98	36.436	3.211
2	759.95	70.109	2.017	844.82	696.3	21.876	0.865
3	879.54	72.304	0.867	935.48	844.82	12.426	0.215
4	1029.99	60.847	9.328	1107.14	935.48	30.805	4.677
5	1120.64	66.988	0.684	1232.51	1107.14	19.667	0.054
6	1419.61	68.544	4.164	1519.91	1274.95	37.085	3.271
7	1637.58	70.371	3.478	1874.81	1519.91	47.719	2.438
8	2928.87	65.36	2.218	2995.45	2426.45	89.07	1.31
9	3365.78	52.128	15.325	3720.69	2995.45	166.877	42.784



	Peak	Intensity	Corr. Intensity	Base (H)	Base (L)	Area	Corr. Area
1	432.05	39.93	0.221	453.98	405.12	11.766	0.032
2	530.42	32.082	1.686	642	443.83	43.848	0.472
3	574.79	28.759	3.061	596	543.93	26.846	1.089
4	881.11	58.027	5.851	908.47	886.04	9.767	0.817
5	1041.56	6.166	8.954	1060.85	933.55	97.759	14.953
6	1078.21	6.529	4.369	1136.07	1062.78	69.601	5.103
7	1157.29	18.081	11.142	1190.08	1138	32.047	4.707
8	1251.65	39.61	3.476	1210.01	1192.01	10.283	0.491
9	1281.8	42.236	4.141	1278.81	1219.01	21.252	1.225
10	1371.39	34.041	7.379	1396.46	1328.95	28.372	2.711
11	1421.54	38.03	5.883	1527.62	1398.38	40.618	3.722
12	1643.35	46.145	23.708	1806.03	1563.56	44.526	9.519
13	2920.23	37.057	29.839	2989.31	2360.87	98.92	32.644
14	3381.21	9.838	58.57	3822.91	3001.24	475.005	329.97

Figure 2. Infered spectera of β-glucan standard (a) and extracted from S. boulardii (b).

Biological experiment

Experimental animals

The treatments of group A1 rats fed on standard diet and group A2 fed on β-glucan solution showed no abnormal behavior or pathological signs throughout the 30-day dosing period in the precent study. Also, when the rats were dosed with β-glucan, no pathological effects or abnormal behavior were observed, no hair loss was observed, and there was no toxicity of the substance during the dose [45] [46].

Weights of experimental rats

There were no significant difference in the average weights after the treatment of rats

between the two groups, the control group (A1) and the treatment group A2. However, the average weight differences of the treatment were significantly lowest compared to the control after 30 days (Table 1).[41][42]

The group fed on beta-glucan showed an increase in weight, but less than the control group A1, 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 eaten and delays the feeling of hunger, and then reduces the amount of energy that it supplies. body, resulting in weight loss [43]. Emerging evidence indicates that insoluble dietary fiber (β-glucan) prevents obesity by regulating intestinal absorption. [44].

Table (1) Measurment of animal weight (g) before and after 30 dosing days of rats with and without β-glucan treatments.

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**
β-Glucan	19.64 b	**36.0 b**	**219.0 a**	**183.0 a**
LSD	**1.27	**2.31 ** **	**4.69 NS**	**3.74 NS**

NS: non-significant, star *, ** means significant differences at the level 0.05. similar letter menas no significant differences.

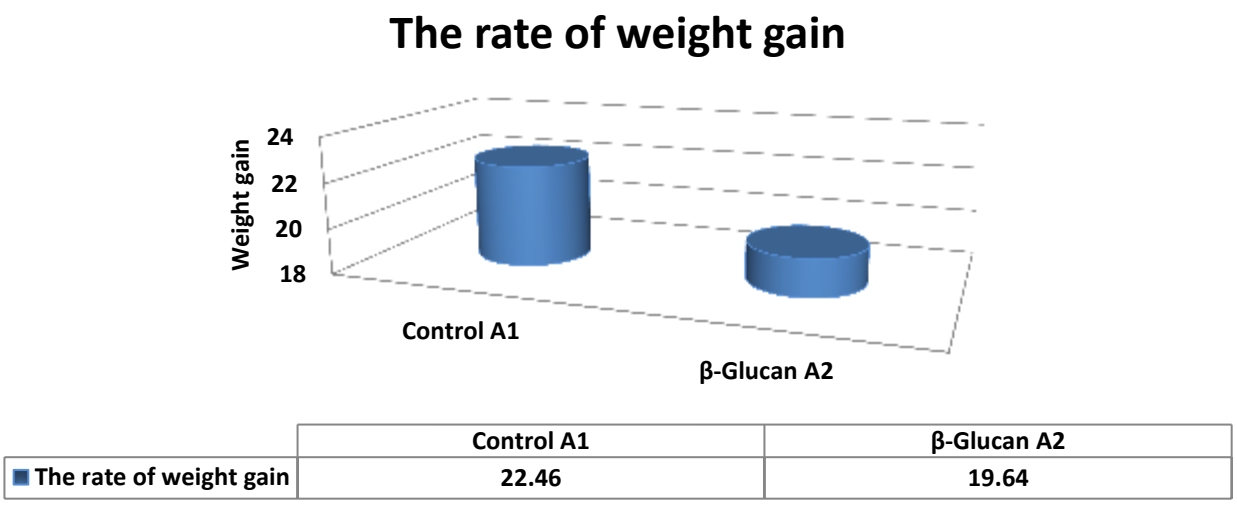


figure (2) shows the rate of weight gain for treatment A1 and A2

Average weights of some internal organs of mice

The results showed that there were no statistically significant differences in the mean weights of the liver and spleen in the rats treated with beta-glucan compared to the comparison treatment. However, rates of treated kidneys increased (Table 2). A small increase in liver

weight is associated with the ability of β-glucan to improve the gut ecological balance of experimental animals by competing with pathogenic bacteria for nutrients in the digestive tract. In addition to its role in improving the absorption of raw materials for nutrients available in the small intestine, and its production of some types of vitamins and some compounds that remove toxic effects and short-chain fatty acids. Especially butyric acid, the main energy source for epithelial cells, and its intervention in enhancing the production of some growth factors that affect the reactivation of epithelial cells, which leads to a balanced increase in treated body weight compared to A1 control group [45] As for the spleen, the results showed no There were statistically significant differences at the level (P<0.05), where it was for (A2) - β-glucan 1.26 g, which is higher than the control level. Group (A1) of 1.14, but no significant effect appeared. As for the weights of the kidneys, the results showed that there were significant differences at the level (P<0.05), where the weight of the kidneys was 2.16 g. Group A2, which is higher than the control group (A1), which was 1.60 g.

Average weight of som internal organs (g)

Table (2) The average weights of the some internal organs .

Treatments	**Average weight of som internal organs (g)**					
	Liver		**Spleen**		**Kidneys**	
Control A1	**b**	**1.60**	**a**	Control A1	**a**	**8.72**
β-Glucan A2	**a**	**2.16**	**a**	β-Glucan A2	**a**	**9.06**
Significant t-test	**0.001*** **		**0.067** **N.S**		**0.437** **N.S**	

Similar letters indicate that there are no significant differences, and different letters indicate that there are significant differences. G.M: Not significant - *, **, *** Significant at the level of 0.05, 0.01 , 0.001,

Immunological tests

Measurement of the level of the immune cytokine interleukin-10

The results of the immune cytokine IL-10 response of the extracted β-glucan from *Saccharomyces boulardii* using ELISA method of showed there was a statistically significant increase in the level of IL-10 concentration after dosing mice with beta-glucan (A2) extract (Table. 3 and Fig .3). Compared with a value of 133.07 pg/ml for the control treatment A1 , a significant and highest value of 152.78 pg/ml were recorded for the treatment A2. It has been recorded that β-glucan in the gastrointestinal tract has a strong effect in stimulating and increasing the immune response. These results indicated that β-glucan may abile to bind to the special receptors represented by TLR-2 and Toll Like Receptor-2 and the Dectin-1 receptor and other receptors stimulating immune cells to produce anti-inflammatory cytokines [46]. and

the effect of β-glucan can be direct in stimulating immunity by binding to the Dectin-1 receptor without the need for (TLR-2), which ultimately leads to enhancing IL-10 production [47].The interaction between dendritic cells (DCs) and β-Glucan is partially dependent on Dectin-1 and promotes elevated IL-10 production by T cells.

Table (3) shows the effect of the two treatments on interleukin production and the effect of the two treatments on the secretion of IgE, IL10 and IgM

Dosing type	pg/ml IL		IgM ml/mg		IgE ml/ng	
Control A1	**b**	**133.07**	**b**	**1.620**	**a**	**116.2**
β-Glucan A2	**a**	**152.78**	**a**	**2.344**	**a**	**120.0**
Significant t-test	****0.001**		**0.003 ****		**0.329 N.S**	

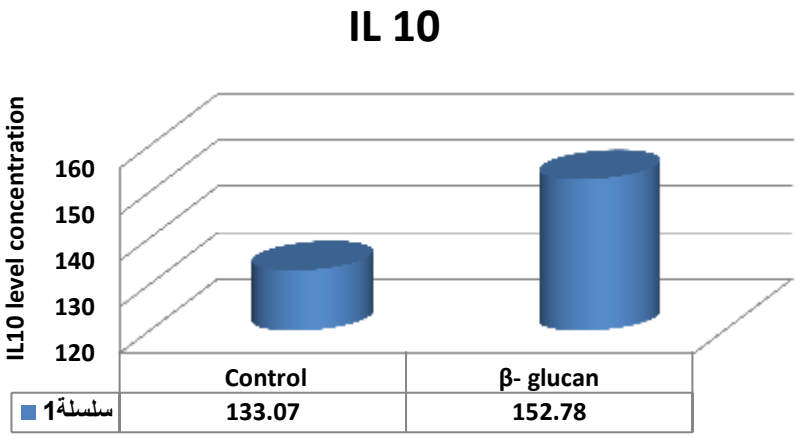


Figure (3) shows the effect of the three treatments on interleukin production

Effect of the selected isolates on the level of antibodies:

IgM assay

The presented results in the Table (3) and Figure (4) of measuring IgM levels using the ELISA method showed there were significantly differences in the level of IgM after dosing mice with beta-glucan compared to the control treatment (A1. The highest value of IgM was 2.344 mg/ml for the A2 treatment, while the value of IgM was 1.620. mg/ml in the control treatment (A1) . These results may be suggested that beta-glucan involved in the production of IgM antibodies and triggered an antigen-specific response [48].

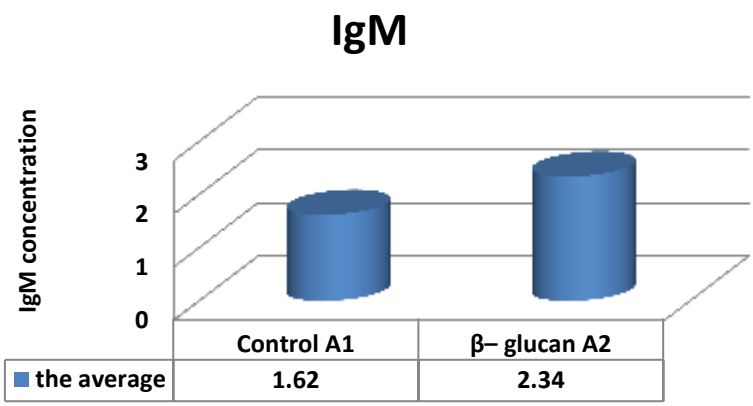


Figure (4) The effect of the two treatments on IgM secretion

IgE screening

The current results shown in Table (3) and Figure (5) of measuring IgE levels using the ELISA method showed there was no statistically increase in the level of IgE after dosing mice with beta-glucan A2 (Fig. 5). Oral administration of beta-glucan decreased IgE concentration levels and was low enough that there were no adverse reactions attributable to the co-administration of beta-glucan in the clinical trial. That beta-glucan has immunomodulatory properties when taken orally or by injection [49], it was found that beta-glucan given orally is less allergic than that given by injection. It was found that beta-glucan did not cause histamine release at any concentration. [50] Beta-glucan improves the immune response [51]

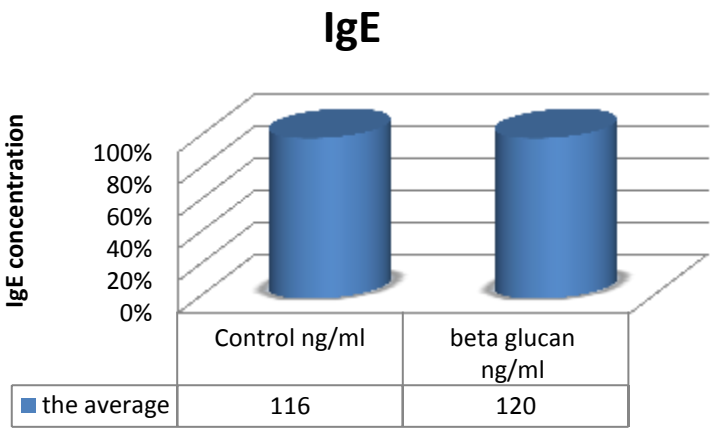


Figure (5) shows the effect of the three treatments on IgE secretion

Conclusions:

In the current paper, beta-glucan was extracted from the cell wall of S.boulardii . The extraction method was useful for obtaining beta-glucan with providing a good quantity and purity of the extract after applying HPLC and FTIR analyses.

concentration of yeast cells and biomass development using vibrating cultures and YPD liquid medium, and extracting beta-glucan by

acid-base method, as it turned out to be the optimal method, and phosphoric acid was used for the purpose Extract purification and from the results of the research, it was found that the beta-glucan extracted in this way gave a larger amount, and purification with acid gave a positive result, by verifying the purity of the extract by HPLC and FTIR examination, and comparing the results with the control. It was found that beta-glucan gave a positive result in the immunoassays IL10 and IgM compared to the control treatment. there was no significant change shown regarding the IgE test. This suggests that β-glucan is a safe substance and does not raise allergies.. While the rats that were dosed with the extract did not show any significant increase in the final weight of the rats. Because beta-glucan is a fiber that helps increase immunity and helps feed beneficial bacteria in the intestine.

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