European Journal of Clinical Pharmacy
Print ISSN: 2385-409X, **Online ISSN**: 3105-0409
Website: https://farmclin.com/

Effect of Mannan extracted from Yeast *Saccharomyces boulardii* on Biochemical Indicators of Laboratory Animals with induced Hyperlipidemia

Article History:

Received: 12-03-2026
Revised: 16-03-2026
Accepted: 22-04-2026
Published: 26-04-2026

Name of Author:
Wael Mahdi Salih [1], and Athraa Harjan Mohsen[1]

Affiliation: *[1]Department of Biology, Faculty of Science, University of kufa, 54001, Iraq*
[1]Department of food science, Faculty of Agriculture, University of kufa, 54001, Iraq
Waelm.alameri@student.uokufa.edu.iq , athraa.alduhaidahawi@uokufa.edu.iq

Corresponding Author:

Abstract: Background: Because hyperlipidemia resulting from high-density diets is a disorder, and also because of the risks it poses to the heart, and given the blood disorders, This study aims to assess the lipid-lowering with hepatoprotective properties of extracted mannan from the yeast Saccharomyces boulardii in rats affected by induced hyperlipidemia . Thirty two male white rats (Rattus norvegicus) were used in the study and divided into eight caloric groups. Hyperlipidemia was induced by feeding the animals a high-density diet, and then different doses of mannan were administered via visual dosing at doses of 25, 50, 75, and 100 mg/kg of body weight. Blood lipid profile markers were measured: (TC), (TG), (HDL-C), (LDL-C), (VLDL-C), along with (ALT), (AST), and (ALP).The high-fat diet group (G2) showed elevated levels of TC (194.0 mg/dL), TG (85.8 mg/dL), LDL-C (154.4 mg/dL), VLDL-C (17.15 mg/dL), and liver enzymes ALT (60.25 U/L), AST (110.5 U/L), and ALP (170.2 U/L), along with decreased HDL-C (22.5 mg/dL) compared to the control group (G1).Mannan treatment improved these parameters in a dose-dependent manner. The 25 mg/kg dose (G3) showed slight improvements, while 50 mg/kg (G4) and 75 mg/kg (G5) doses produced progressively greater reductions in TC, TG, LDL-C, VLDL-C, and liver enzymes, with increases in HDL-C; the 75 mg/kg dose showed the most pronounced effect (TC 140.0, TG 72.8, LDL-C 79.2, HDL-C 46.25). The 100 mg/kg dose (G6) showed less improvement than G5. The Atorvastatin group (G7) also showed significant improvement, and the standard diet plus 100 mg/kg mannan group (G8) nearly matched control values, indicating that mannan effectively improves lipid profile and liver function, with an optimal dose at 75 mg/kg. These results indicate that mannan extracted from the wall of S. boulardii yeast has lipid-lowering and hepatoprotective effects in rats fed a high-fat diet with the best therapeutic response at a dose of 75 mg/kg of body weight.

Keywords: Mannan; Saccharomyces boulardii; Dyslipidemia; High-fat diet; Lipid profile; Hepatic enzymes; Functional food; Albino rats.

INTRODUCTION

Congenital melanocytic nevi (CMN) are pigmented Metabolic diseases, especially hyperlipidemia, have emerged as increasingly common health issues worldwide due to alterations in nutrition and reduced physical activity. They are directly associated with an elevated risk of cardiovascular illness and metabolic liver dysfunction [1]. This has prompted research into functional food compounds of natural origin that can improve biochemical indicators and reduce reliance on traditional drug therapies. Polysaccharides are bioactive compounds that have demonstrated important roles in regulating metabolism, modulating the gut microbiome, and influencing lipid and blood glucose metabolism [2]. Among these compounds is mannan, a polysaccharides constituting yeast cell

walls and possesses biological properties dependent on its molecular structure and glycosurgical patterns [3]. Adding mannan to food may contribute to lowering total cholesterol and triglycerides and positively impact liver function [4]. Saccharomyces boulardii yeast is extensively utilised in the culinary and medicinal sectors because to its significant biological attributes and the high mannan content of its cell wall. [5]. Despite yeast's significant role in mitigating or addressing numerous diseases, the impact of pure mannan extracted from the cell wall of S. boulardii on biochemical markers in hyperlipidaemia models is still inadequately explored[6].

Therefore, the present work sought to assess the biochemical impacts of mannan derived from the cell wall of S. boulardii yeast when administered orally to rats with high-fat diet-induced hyperlipidemia by examining its effect on blood biomarkers and liver function, in order to explore its potential as an independent natural functional compound.

MATERIALS AND METHODS

Experimental Animals

In this investigation, a total of 32 male albino rats of the Wistar (Rattus norvegicus) were used. The animals underwent experimental treatments that included oral administration of mannan previously extracted from the wall of *S. boulardii* yeast at different doses according to the experimental design of the study. The animals were 10 weeks old with an initial weight ranging between 120–150 g and were housed in standard laboratory conditions at a temperature of 22±2°C with a 12-hour light/12-hour dark lighting cycle, and free access to water and feed. All procedures were carried out in accordance with the Guide for the Care and Use of Laboratory Animals [7]and in compliance with the approved ethical standards.

Experimental Design

The animals were allocated randomly into eight groups (n = 4 per group) using a completely randomized design (CRD). Hyperlipidemia was established by administering a high-fat meal to the animals for one month previous to the treatment intervention, a conventional model for investigating lipid metabolism disorders in rats [8, 9].

Groups were assigned as follows

G1: Standard diet + 0.9% physiological saline (NaCl) dosing (negative control).

G2: High-fat diet (positive control).

G3: High-fat diet + 25 mg/kg mannan.

G4: High-fat diet + 50 mg/kg mannan.

G5: High-fat diet + 75 mg/kg mannan.

G6: High-fat diet + 100 mg/kg mannan.

G7: High-fat diet + atorvastatin (10 mg/kg).

G8: Standard diet + 100 mg/kg mannan.

Treatment with mannan or atorvastatin began during the second month, while continuing the prescribed diet for each group, and the intervention period lasted four weeks. Mannan, isolated from the cell wall of *S. boulardii* yeast, was administered orally via a gastric tube daily in doses determined according to body weight. Atorvastatin was supplied identically to the controlling group, while the first group received a saline solution.

Biochemical Assessment

2.3.1 Estimation of Total Serum Cholesterol

Samples of blood have been obtained at the conclusion of the study period following an overnight fast. To alleviate discomfort in the rat, it was administered an intramuscular injection of 0.25 ml/100g of xylazine and 5 mg/100g of ketamine. Blood samples were obtained from all groups utilizing cardiac pricking employing 10 ml medical syringes. To measure physiological parameters, blood samples were allocated to tubes with anticoagulant, while the remaining samples were placed in test tubes without anticoagulant and allowed to stand for 10-15 minutes at laboratory temperature. Then the sample was centrifuged at a speed of 3000 rpm to separate the serum from the rest of its components. The serum was separated and placed in tubes for the purpose of conducting biochemical tests [10].

2.3.2 Estimation of Serum Triglycerides

Yoshino *et al.* (1992)[12] detailed an enzymatic colorimetric approach for estimating serum triglyceride (TG) concentrations using commercially available diagnostic kits. The process depends on lipase's enzymatic hydrolysis of triglycerides to generate glycerol, which is then oxidized to form hydrogen peroxide by glycerol oxidase. In the presence of peroxidase, a colored complex is formed when hydrogen peroxide reacts with the colorimetric reagent. A spectrophotometer was used to detect the absorbance at 550 nm. The serum triglyceride concentration was then determined by comparing the result to a standard solution.

Estimation of High-Density Lipid-C Concentration in Serum

According to [13], a chemical precipitation method was used to measure the serum concentration of high-density lipoproteins (HDL-C). By utilizing phosphor-tungstic acid in the presence of magnesium ions, this approach separates HDL-C from the filtrate and precipitates low-density lipoproteins (LDL) and chylomicrons. The filtrate's cholesterol concentration was ascertained following centrifugation by means of an enzyme-chromatographic method; the absorbance was quantified at a 500 nm wavelength by means of a spectrophotometer. After that, it was compared to a reference solution in order to determine the HDL-C concentration.

Calculation of Low-Density Lipoproteins in Serum
To find LDL, the equation from [14] was utilized. This math's is:
LDL-Cholesterol (mg /DL) = Total cholesterol – (VLDL + HDL)

2.3.5 Estimation of Liver Enzyme Activity in Serum: Alanine and Aspartate Transaminases (ALT, AST)-ALP
Based on pre-prepared diagnostic kits from BioMérieux, France, the standard colorimetric approach as reported by [15] was used to measure the activity of the liver enzymes Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), and Alkaline phosphatase (ALP) in serum. The method relies on measuring the formation of enzyme reaction products such as pyruvate and oxaloacetate, which result from the activity of ALT and AST enzymes and react with 2,4-dinitrophenylhydrazine to form colored compounds that can be measured spectroscopically. ALP enzyme activity was estimated using a colorimetric enzymatic method with a suitable substrate, and its absorption was assessed at 540 nm utilizing a spectrophotometer. The findings were presented as a unit of U/L.

Statistical Examination
Utilizing GenStat software, the data underwent statistical analysis. Within a CRD, a one-way analysis of variance (ANOVA) was implemented. According to the recommendation for analyzing data from biological experiments [16], Duncan's Multiple Range Test was utilized to compare means at a significant level of $P < 0.05$ when significance variances were discovered across treatments.

RESULTS AND DISCUSSION

Distribution of Study Samples by Gender
Table 1 shows the effect of mannan isolated from the cell wall of S. boulardii yeast at different doses on serum lipid indices (TC-TG-HDL-LDL-VLDL) and liver function enzymes (ALP-AST-ALT) in rats with high-fat diet-induced hyperlipidemia. Values are reported as means for each experimental group following the four-week treatment period.

Table (1) Effect of mannan isolated from the cell wall of S. boulardii yeast on biochemical indicators in rats

Group	TC	TG	HDL	LDL	VLDL
G1(control-)	a **82.2**	a **41.5**	bc **35.75**	a**38.2**	a **8.30**
G2 (Control+)	c **194.0**	d **85.8**	a **22.50**	d **154.4**	d**17.15**
G3 (25 mg/kg)	c **183.0**	cd **84.5**	a **24.50**	d**141.6**	bcd **16.90**
G4(50 mg/kg)	b **155.0**	cd **83.2**	bc **36.50**	c**101.8**	cd **16.65**
G5(75 mg/kg)	b **140.0**	b **72.8**	d **46.25**	b **79.2**	b **14.55**
G6(100 mg/kg)	c **196.2**	bcd**81.2**	c **38.75**	d **141.2**	bcd **16.25**
G7(Atorvastatin)	b **140.5**	bc**75.2**	b **32.75**	bc **92.7**	bc **15.05**
G8(100mg/kg+normal diet)	a **64.0**	a **33.2**	b **32.50**	a **24.9**	a **6.65**

* Distinct letters within the same column signify significance variations at a significant level for $P < 0.05$, as determined by Duncan's multiple range test.

The finding for two tables indicate that feeding on the high-fat diet led to a clear disturbance in blood lipid indicators and liver functions in laboratory rats. The second group fed on the high-fat diet showed a significance increases in (TC), (TG), (LDL-C), and (VLDL), with values of 194.0, 85.8, 154.4, and 17.15 mg/kg respectively, compared to the first group fed on the standard diet with 0.9% physiological saline dosing, which recorded 82.2, 41.5, 38.2, and 8.30 mg/kg respectively. In contrast, the concentration of high-density lipoprotein (HDL-C) decreased in the high-fat diet group to 22.50 mg/kg, compared to the negative control group which recorded 35.75 mg/dL. A significant increase in the liver enzymes ALT, AST, and ALP was also observed in the second group, reaching 60.25, 110.50, and 170.2 U/L, respectively, in contrast to the first group that documented 42.25, 78.50, and 110.5 U/L. The results demonstrate the effective establishment of a hyperlipidemia model induced by a high-fat diet, corroborated by multiple studies indicating that such diets significantly elevate blood lipid levels, promote hepatic fat formation, and induce metabolic abnormalities in experimental rats [8, 9].

Table 2 Effect of mannan extracted from the wall of S. boulardii yeast on liver enzymes (ALT-AST-ALP)

Group	ALT	AST	ALP
G1(control-)	42.25 a	78.50 a	110.5 a
G2 (Control+)	60.25 c	110.50 f	170.2 a
G3 (25 mg/kg)	58.00 c	103.25 e	167.0 d
G4(50 mg/kg)	51.25 b	93.50 c	132.5 b
G5(75 mg/kg)	45.75 a	87.50 b	161.2 cd
G6(100 mg/kg)	50.50 b	97.50 cd	161.2 cd
G7(Atorvastatin)	52.50 b	99.75 de	156.0 c
G8(100mg/kg+normal diet)	43.50 a	81.50 a	112.5 a

* Distinct letters within the same column signify substantial variations at a significant level of $P < 0.05$, as determined by Duncan's multiple range test.

The mannan-treated groups exhibited substantial enhancement in the majority of biochemical parameters relative to the control group subjected to the high-fat diet. A 25 mg/kg dosage (G3) led to a marginal reduction in total cholesterol to 183.0 mg/kg and LDL-C to 141.6 mg/kg, accompanied by a modest enhancement in HDL-C to 24.50 mg/kg. A 50 mg/kg dosage (G4) demonstrated a more significant enhancement, with total cholesterol decreasing to 155.0 mg/dL and LDL-C to 101.8 mg/dL, while HDL-C increased to 36.50 mg/dL. Liver enzymes ALT, AST, and ALP also decreased to 51.25, 93.50, and 132.5 U/L, respectively, compared to the control group. This improvement reflects the potential effects of bioavailable polysaccharides in regulating lipid metabolism, as studies indicate that these compounds can improve cholesterol reverse transport and regulate gene expression of membrane transporters associated with cholesterol excretion such as ABCA1, in addition to their role in activating some metabolic pathways regulating lipid metabolism such as PPARα receptors [2]. The 75 mg/kg (G5) dose achieved the best physiological response among the mannan doses, with total cholesterol decreasing to 140.0 mg/kg and triglycerides to 72.8 mg/kg, while LDL-C decreased to 79.2 mg/kg and VLDL to 14.55 mg/kg. HDL-C, on the other hand, increased to 46.25 mg/kg. Liver enzymes ALT and AST also decreased to 45.75 and 87.50 U/L, respectively, compared to the control group. This lipid-lowering effect can be explained by mannan's ability to bind to bile salts in the intestine and reduce their reabsorption, leading to increased hepatic cholesterol consumption for the production of new bile acids, thus lowering blood cholesterol concentration [17]. One possible mechanism by which changes to the gut microbiome and an increase in the synthesis of short-chain fatty acids can activate the AMPK pathway, that regulates metabolism of energy, and so suppress fat formation in the liver [18].

Although total cholesterol climbed to 196.2 mg/dL and LDL-C to 141.2 mg/dL at the maximum dose of 100 mg/kg of mannan, the response was less dramatic than at the typical doses, suggesting that higher doses do not always result in better bioavailability. Reason being, best responses are seen at moderate doses rather than high ones, which is consistent with a non-linear dose-response relationship seen in many biologics. As per [2], this phenomenon is referred to as the inverted U-shaped reaction. The atorvastatin group showed a marked improvement compared to the control group fed a high-fat diet. Total cholesterol decreased to 140.5 mg/kg, triglycerides to 75.2 mg/kg, LDL-C to 92.7 mg/kg, VLDL to 15.05 mg/kg, and HDL-C to 32.75 mg/kg. Liver enzymes ALT, AST, and ALP also decreased to 52.50, 99.75, and 156.0 U/L, respectively, compared to the control group.

This findings corroborate the hypothesis that HMG-CoA reductase inhibitors, like atorvastatin, enhance lipid profiles and decrease the likelihood of cardiovascular events by lowering cholesterol levels and decreasing the generation of (LDL) in the liver [19]. The eighth group, which received mannan at a dose of 100 mg/kg with the standard diet,

showed values close to those of the first group: total cholesterol 64.0 mg/kg, triglycerides 33.2 mg/kg, LDL-C 24.9 mg/kg, VLDL 6.65 mg/kg, and HDL-C 32.50 mg/kg. Liver enzymes were also recorded at 43.50, 81.50, and 112.5 U/L for ALT, AST, and ALP, respectively, values close to those recorded in the negative control group. These results suggest that mannan supplementation within a standard diet does not cause disturbances in biochemical indicators and may even contribute to maintaining normal metabolic balance.

In general, the results indicate that mannan isolated from the yeast S. boulardii has a clear effect in improving lipid profiles and reducing liver function disturbances resulting from feeding on a high-fat diet, with the best response achieved at a dose of 75 mg/kg, which confirms the importance of determining the optimal biological dose to achieve the best physiological effect

CONCLUSION

This study demonstrates that mannan extracted from the cell wall of the yeast S. boulardii significantly enhances biochemical markers related to hyperlipidemia caused by a high-fat diet in rats. The high-fat diet resulted in a substantial elevation of total cholesterol, triglycerides, LDL, and VLDL, accompanied by a reduction in HDL and an increase in liver. enzymes, confirming the successful induction of a hyperlipidemia model associated with hepatic metabolic stress. Mannan treatment demonstrated a clear improvement in blood lipid indicators and liver function compared to the untreated high-fat diet group. This impact varied with dosage within a specific range. A drop in total cholesterol, LDL-C, VLDL, and triglycerides, an increase in HDL-C, and a significant improvement in the liver enzymes ALT, AST, and ALP were all observed at the 75 mg/kg dose, which also produced the best physiological response. On the other hand, there seems to be an ideal dosage range for biological response, since the greater dose of 100 mg/kg had no further effect on efficacy. The effectiveness of the experimental model was further supported by the fact that blood lipid parameters in the atorvastatin group improved significantly when compared to the untreated control group. Biochemical readings remained within normal physiological ranges following administration of 100 mg/kg of mannan with the conventional meal, suggesting that the molecule was safe and had no negative impact on normal metabolic state. In sum, our findings point to the possibility that Saccharomyces boulardii mannan has useful functional characteristics for reducing metabolic stress in the liver and enhancing blood lipid balance in response to a high-fat diet, with the most effective dose being moderate. To better understand the bioactive pathways of this chemical, however, further research utilizing more comprehensive biological models and molecular mechanism analysis is required. There was a modest improvement in liver enzymes: ALT to 52.50, AST to 99.75, and ALP to 156.0 in the atorvastatin group. HDL climbed to 32.75 and VLDL to 15.05. TC, LDL, and TG all decreased to 140.5, 92.7, and 75.2, respectively. There were no noticeable side effects on liver enzymes in the eighth group, which consisted of the usual diet plus 100 mg/kg mannan, and whose readings were within physiological limits: TC at 64.0, TG at 33.2, and LDL at 24.9. These findings suggest that *S. boulardii* mannan has hepatoprotective and lipid-lowering effects in high-fat diets, with 75 mg/kg being the best dosage for therapeutic efficacy.

Acknowledgments

The authors would like to express their sincere gratitude to the 1Department of Biology, Faculty of Science, University of kufa, Iraq, for providing the laboratory facilities and scientific support necessary to accomplish this study.

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