RESEARCH ARTICLE

Saccharomyces boulardii, a novel yeast probiotic ameliorates metabolic syndrome associated with PCOS: A preliminary study

Sananda Sil[1], Angshita Ghosh[1], Tarun K. Kar[1], Suman K. Halder[2], Sandip Chattopadhyay[1*]

ABSTRACT

Background: Polycystic ovary syndrome (PCOS) is one of the most common yet very complex endocrinopathies in women of reproductive age accompanied by metabolic alteration. Gut microbiota has been found to play a critical role in the pathophysiology and further progression of PCOS. The wide range of side effects of existing medical management have made it necessary to continuously look for an efficient and safe alternative. *Saccharomyces boulardii,* the only eukaryotic probiotic with its unique properties is becoming very popular as the only yeast probiotic. *Hypothesis:* This preliminary study evaluated the efficacy of *S. boulardii* on a PCOS-IR (PCOS with insulin resistance) rat model. *Materials and methods:* PCOS was induced in all virgin female Wistar rats with 1 mg/kg/day of letrozole and high fat (40%) diet except for the control group for 21 days. The rats were then co-administered with lyophilized *S. boulardii* of 1.8×10^7 CFU /kg/Day. *Results: S. boulardii* prevented the further progression of PCOS by restoring the ovarian morphology and metabolic parameters. *Conclusion:* However, this mechanism can be attributed by the possible correction of dysbiosis and metabolic alteration in PCOS by *S. boulardii*.

Keywords: Polycystic ovary syndrome, *Saccharomyces boulardii,* Insulin resistance, Letrozole, Gut microbiota, Probiotics, Metabolic syndrome.

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INTRODUCTION

Polycystic ovarian syndrome (PCOS), the heterogeneous endocrine-metabolic condition with a multifaceted pathogenesis, has long-lasting impacts on women's health that go far beyond the reproductive years.[1] Depending on its diagnostic criteria Worldwide, 6 to 20%,[2] and 3.7 to 22.5% Indian women of reproductive age are suffering from PCOS.[3] Core features of PCOS are clinical and/or biochemical hyperandrogenism (HA), ovulatory dysfunction (OD), and polycystic ovarian morphology (PCOM).[4] PCOS is associated with multiple morbidities, including reproductive abnormalities and infertility, endometrial cancer, non-alcoholic fatty liver disease (NAFLD) and depression.[5] Recently, the dysmetabolic component of PCOS has drawn a lot of attention.[6] Insulin resistance (IR) seems to play a role in the etiology of PCOS and consequent metabolic syndrome, which includes impaired glucose tolerance, overweight or obesity (particularly, increased visceral fat), dyslipidemia, and cardiovascular morbidity.[7] Although the exact cause of PCOS is still unknown, it is thought to be the result of a complex interaction of metabolic, endocrine, genetic, behavioral, and environmental variables that result in an imbalance of hormones and metabolism.[8,9] Numerous studies linking the gut microbiota to the development of metabolic disorders have led researchers to postulate that changes in the microbiome may also cause PCOS.[10,11] According to the Dysbiosis of Gut Microbiota (DOGMA) hypothesis, increased intestinal permeability caused by an imbalanced gut flora could lead to lipopolysaccharide (LPS) leaking into the systemic circulation. Consequently, the immune system is activated, resulting in an inflammatory response and ultimately IR.[12]

[1]Department of Biomedical Laboratory Science & Management, Vidyasagar University, Midnapore, India.

[2]Department of Microbiology, Vidyasagar University, Midnapore, India.

***Corresponding author:** Sandip Chattopadhyay, Department of Biomedical Laboratory Science & Management, Vidyasagar University, Midnapore, India, Email: sandipdoc@mail.vidyasagar.ac.in

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Evidence suggests that probiotics can treat PCOS by reducing insulin resistance, inflammation, and oxidative stress by correcting sex hormone binding globulin (SHBG) levels, dyslipidemia and obesity.[13] *Saccharomyces cerevisiae* var. *boulardii* (*S. boulardii*), the most extensively studied probiotic yeast, has previously been popular for its use in prevention of antibiotic-associated diarrhoea.[14] However, recent studies have found a promising role of *S. boulardii* against viral infections, liver diseases, and metabolic disorders associated with cardiometabolic risk factors, oxidative stress and inflammation.[15-19] The beneficial effect of *S. boulardii* have been linked to a variety of mechanisms, including specific antitoxin effects, antimicrobial activities, a trophic effect on the gut mucosa, an enhanced immune response, and increased production of the short-chain fatty acid (SCFA), butyrate.[14,20] SCFA is closely linked to gut health, insulin sensitivity and metabolic syndrome.[21]

Although the therapeutic efficacy of *S. boulardii* on various health conditions is well demonstrated by several studies, the probiotic yeast *S. boulardii* has never been investigated till date in the context of PCOS. In the present study, we investigated the therapeutic potential of *S. boulardii* on PCOS-IR animal model, as well as its role in the modulation of metabolic parameters and oxidative stress markers.

MATERIALS AND METHODS

Animal Selection and Care

For the investigation, 18 virgin female albino wistar rats were used. They were kept in clean polypropylene cages in three divisions, having six each under standard laboratory settings at 23 ± 2°C and 12-h light and dark cycles throughout the experimental period, receiving food and water ad libitum. The entire experiment was conducted in accordance with a valid institutional ethical clearance approved by the Institutional Animal Ethics Committee (IAEC).

Experimental Design

Eighteen animals were randomized to three groups (n = 6 in each group). Rats were treated by oral gavage once daily for 21 days according to the following group distribution.

- Group I (Control): The control group was vehicle-treated fed with normal rat chow diet (Table 1).
- Group II (PCOS-IR): The treated group received letrozole 1mg/kg BW/Day dissolved in carboxy methyl cellulose (CMC) and high fat (40%) diet (Table 1).
- Group III (PCOS-IR+Sb): The supplement group was co-administered with lyophilized *Saccharomyces boulardii* 1.8×10^7 CFU /kg /Day[22] along with letrozole (1mg/kg BW/Day) in CMC vehicle and fed with high fat (40%) (Table 1). Commercial formula of *S. boulardii* CNCM I-745 ('Econorm' by Dr. Reddy's laboratories Ltd.) was used with 12×10^9 viable cells/gram of sachet content.

On day 22, serum and relevant organs were collected from euthanized rats and stored at 20°C for further experiment.

Measurement of body weight and length of the rats

The body weight of rats was measured, at the beginning and on the completion of experiment, difference in weight were measured. Lengths of the rats were measured assuming a straight line from nose to anus at the end of the experiment. The Lee's index was calculated using the formula-

$$\text{Lee Index} = (\text{weight in gm} \times 1000)^{1/3}/\text{length in cm}$$[23]

Serum Metabolic Profile

Fasting blood glucose (FBG) and lipid profile (total cholesterol (TC), triglycerides (TG), and high-density lipoprotein cholesterol (HDL-C)) were assessed using commercial kits, in accordance with the set recommendations. The following formulas were used to estimate levels of very low-density lipoprotein (VLDL-C), low-density lipoprotein cholesterol (LDL-C), coronary risk indices, atherogenic index, and triglycerides-glucose index:

- VLDL-C (mg/dl) = Triglycerides / 5[24]
- LDL-C (mg/dl) = Total cholesterol – (HDL-C + VLDL-C) (22)
- The coronary risk indices= (TC / HDL-C and TG / HDL-C ratio).[25]
- Triglyceride-glucose index (TyG index) = Ln [TG (mg/dl) × FPG (mg/dl)/2][26]

Table 1: Nutritional composition of different diets given to experimental rat

Normal rat chow diet			
Ingredients	*Quantity (g/ 100g)*	*Nutrients (%/100g)*	
Whole Wheat flour	57	Carbohydrate	69
Roasted Bengal gram flour	32	Protein	18
Whole Milk powder	10	Fat	13
Soybean oil	1	Total energy (Kcal)	367
High fat diet			
Ingredients	*Quantity (g/ 100g)*	*Nutrients (%/100g)*	
Normal rat feed	70	Carbohydrate	47
Hydrogenated fat (dalda)	7	Protein	13
Corn oil	6	Fat	40
Milk powder	17	Total energy (Kcal)	450

Ovarian Histopathology

The ovaries were collected from experimental rats after sacrifice, then they were stored in 10% formaldehyde solution for histological study. This was followed by a series of dehydration and rehydration procedures. The tissues were then parafinized, and 5 µm thick tissue sections were dissected using a microtome (Leica, Germany). Sections were viewed under a compound microscope after being stained with hematoxylin and eosin (Sigma Aldrich) (Olympus, CX21i, magnification x 400).

Statistical Analysis

The statistical analysis of present study was performed using SPSS software 16.0. The post hoc Dunnett test was used to assess the statistical significance of the changes in these variables between treated and control groups. Differences of data (Mean ± SEM, N = 6) with $p < 0.05$ were considered statistically significant.

RESULTS

Body weight and Organo-somatic indices

The data in Figure 1 demonstrated a noticeable difference in the final body weight of experimental rat of different groups. However, when compared to the control group, the body weight of rats in the PCOS-IR group significantly

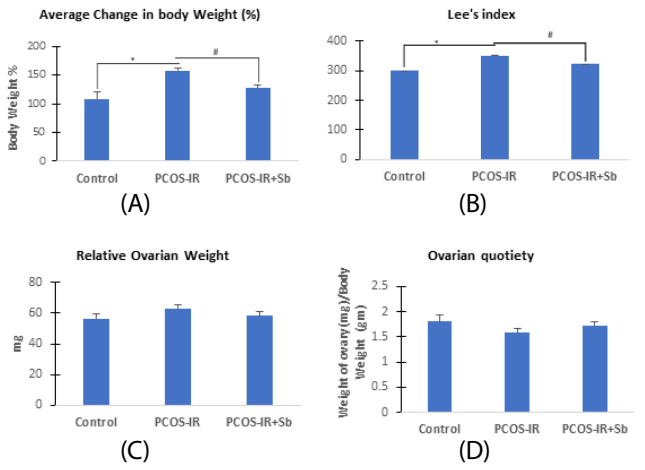

Figure 1: Ameliorative effects of *S. boulardii* on (A) Body weight, (B) Lee's index (C) Ovarian Weight and (D) Ovarian quotiety in rats with letrozole and high fat induced PCOS. Data are presented as the Mean ± SEM (n = 6), evaluated by ANOVA followed by the post hoc Dunnett t (2-sided) test. * and # indicate significant difference in comparison to control and PCOS-IR groups, respectively.

increased (p <0.01). In contrast, supplementation for 21 days with *S. boulardii* caused a substantial reduction in body weight compared to the PCOS-IR group at P <0.05. Although statistically not significant, an increase in the ovarian weight was observed in PCOS-IR group, whereas rats supplemented with *S. boulardii* have shown a decreasing trend towards normalcy.

Serum Metabolic Profile

Data presented in Table 2 showed a significant elevation in levels of fasting plasma glucose (FPG) level in the PCOS-IR group compared to the control group at P < 0.001 while *S. boulardii* supplemented groups showed that FPG level was significantly reduced when compared to the PCOS group at (P < 0.001). Also, lipid profile in PCOS-IR group showed significant alterations as serum total cholesterol (TC), triglycerides (TG), LDL-C and VLDL-C were significantly elevated, while HDL-C showed a significant decrease (P < 0.001) in contrast to the control group. Moreover, *S. boulardii* supplemented groups showed significant (P<0.001) hypolipidemic effect on TC, TG, VLDL and LDL whereas as HDL-C was (P<0.05) found to elevate as compared with the PCOS-IR group. Coronary risk indices (CRI) and triglycerides glucose index (TyG) significantly deteriorated in the PCOS group when compared with the control group at p < 0.001. CRI and TyG were significantly improved in *S. boulardii* treated groups comparable with PCOS group at P < 0.001.

Ovarian Histopathology

The ovarian morphology of experimental rat of different groups were compared to observe the ameliorative effect of *S. boulardii* on the polycystic ovaries of rat. In the normal control group, the follicles in the ovaries of rats were at various stages of development, with several corpus luteum,

Table 2: Total Cholesterol (TC), Triglycerides (TG), High Density Lipoprotein Cholesterol (HDL-C), Low Density Lipoprotein Cholesterol (LDL-C), Very Low-Density Lipoprotein Cholesterol (VLDL-C), Triglyceride Glucose Index (TyG index), Coronary risk index (CRI). Values presented as Mean ± SEM (n = 6), evaluated by ANOVA followed by the post hoc Dunnett t (2-sided) test. * and # indicate significant difference in comparison to control and PCOS-IR groups, respectively.

Parameters	*Control*	*PCOS-IR*	*PCOS-IR+ Sb*
Glucose (mg/dl)	86.95 ± 2.92	178.63 ± 2.44*	127.56 ± 3.12#
TC (mg/dl)	88.25 ± 2.24	148.33 ± 2.75*	107.34 ± 1.5#
TG (mg/dl)	68.72 ± 1.6	126.96 ± 4.92*	84.89 ± 1.68#
HDL-C	48.22 ± 1.71	28.56 ± 1.43*	37.06 ± 1.75#
LDL-C	26.29 ± 2.51	94.37 ± 2.33*	53.30 ± 2.88#
VLDL-C	13.74 ± 0.32	25.39 ± 0.98*	16.97 ± 0.33#
CRI (TC/HDL-C)	1.83 ± 0.07	5.24 ± 0.21*	2.93 ± 0.18#
CRI (TG/HDL-C)	1.43 ± 0.08	4.5 ± 0.30*	2.32 ± 0.14#
TyG Index	7.99 ± 0.04	9.41 ± 0.04*	8.48 ± 0.04#

close and ordered organization of granulosa cells represented a comprehensive morphology, and up to 8 or 9 layers of granulosa cells within the dominant follicle were observed. The ovaries of the PCOS-IR group displayed unorganized ovarian tissue structure with fluid-filled multiple cystic follicles without an oocyte and corona radiata. In addition, it was observed that the follicle contained decreased lutea and typically 2 to 3 layers of granulosa cells. The ovaries from the *S. boulardii* groups showed follicles at various stages of development, with part of the corpus luteum and tight organization of granulosa cells, as well as increased layers (3-7) of granulosa cells within the follicle in comparison to those from the PCOS-IR group (Figure 2).

DISCUSSION

The complicated reproductive condition polycystic ovarian syndrome (PCOS) is linked to multiple hormonal and metabolic alterations.[27] The metabolic manifestation of PCOS, including insulin resistance (IR), obesity, dyslipidaemia and hyperandrogenism, can be categorized as metabolic syndrome.[28] The pathophysiology of PCOS is strongly influenced by increased insulin resistance and compensatory hyperinsulinemia.[29,30] Letrozole (aromatase inhibitor) and high fat diet were used to induce PCOS accompanied with IR in the rats.[31] Our findings supported earlier observations by demonstrating PCOS-like characteristics in rats, including

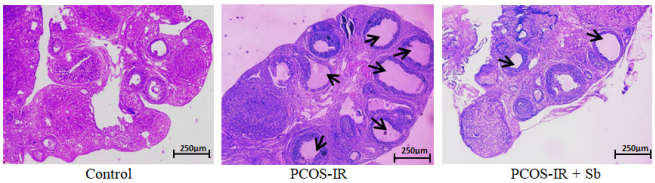

Figure 2: Histopathological features of rats from three groups, (a) Control group (b) PCOS-IR group (c) PCOS-IR+Sb group are presented at 4X magnification. Cystic follicles (CF) are indicated by black arrows

increased body weight, fasting glucose, insulin resistance, and dyslipidemia. To our knowledge this is the first study where *S. boulardii* was used as probiotic yeast to explore its efficacy against PCOS and associated metabolic syndrome in PCOS-IR rat model. The treatment with *S. boulardii* can ameliorate PCOS associated metabolic aberrations in rats by improving insulin resistance and metabolic profile. We also showed that *S. boulardii* has significantly improved ovarian histoarchitecture.

Recent studies found, effectiveness of *S. boulardii* in weight management and fat mass reduction.[17,18] In this present study *S. boulardii* was similarly effective. PCOS-IR group exhibited a significant increase in body weight whereas the *S. boulardii* treated group depicted a significant decrease in body weight, suggesting an anti-obesity effect of this specific yeast in PCOS. Surprisingly, the ovarian weights did not show any significant change.[32] Lee's index was used as a fast and precise technique to determine obesity in rats.[33] Lee's index of PCOS-IR group was significantly higher upon statistical analysis, nevertheless *S. boulardii* supplementation showed an opposite effect.

Several investigations explored the development of early metabolic and cardiovascular risk linked with the PCOS phenotype together with metabolic alterations.[34,35] In accordance with the results of the current investigation, the PCOS-IR group reflected a significant increase in blood sugar, total cholesterol, triglycerides, LDL-C and deteriorating coronary risk markers. These metabolic changes were linked to an increase in the triglycerides-glucose index (TyG), a reliable and relatively acceptable sign of insulin resistance rather than Homeostatic Model Assessment for Insulin Resistance (HOMA).[36-38] This is additionally a good indicator of type 2 diabetes mellitus and coronary heart disease.[37] TyG has been identified as a useful substitute for IR in recent investigations.[40-42] This work used the TyG index as a surrogate marker for IR. Furthermore, IR in PCOS reproductive condition was observed to be significantly correlated with the indices TyG, TG/HDL-C, and TC/HDL-C.[43] This dysregulated lipid and glucose metabolism associated with insulin resistance are suggested to be due to several metabolites produced as a result of gut dysbiosis that ultimately leads to metabolic abnormalities.26 Several studies already reported the efficacy of probiotic bacteria as hypoglycaemic and hypolipidemic.[44,45] In recent findings, the yeast probiotic, *S. boulardii* has been found to correct dyslipidemia both in animal model and in human experiments.[46,47] This yeast probiotic also been reported to exerts its hypoglycemic effect in hyperglycemia.[19,48] In present study, the effect of *S. boulardii* on plasma glucose and lipid profile in PCOS was evident in a similar manner. The protective effect of this yeast probiotic on cardiometabolic parameters can be attributed its probiotic potential and a possible modulation of gut biota. Results of this study were confirmed by histopathological evaluation of the ovarian tissues. *S. boulardii* supplementation showed significant improvement in the ovarian histoarchitecture in comparison with PCOS-IR animal model. Multiple cystic follicles were seen in PCOS-IR group. In this study, insulin resistance measured by TyG index and polycystic morphology of ovary has supported the previous studies that found an association of polycystic ovarian morphology in PCOS with insulin resistance and hyperinsulinemia.[47]

Insulin plays an important role in ovarian steroidogenesis and ovulation[50] through stimulating both granulosa and thecal cells.[41,52] Insulin has a direct stimulatory effect on LH secretion and action.[53] Moreover, Insulin not only acts synergistically with LH in the ovarian theca cells but also it has possible imitates tropic action on theca cells of ovaries,[54] These pathways collectively illustrate the hyperandrogenic scenario imposed on by insulin resistance. The maturation of follicles and the selection of dominant follicles during ova development are both hampered by excessive androgen production in PCOS.[55] Additionally, Insulin and LH work together to arrest the theca cells of the ovaries most often in the preantral and antral stages, which results in the hyperplasia of the theca cells, accumulation of follicular fluid generating cystic formations, impaired ovulation, and ultimately infertility.[56,57] In our study rats with PCOS- IR showed the development of empty cysts filled with follicular fluid could be described by direct and indirect role of insulin resistance. However, the recovered ovarian morphology in *S. boulardii* supplemented group can be attributed to the improved insulin sensitivity along with improved metabolic parameters.

In conclusion, this study demonstrated *S. boulardii* supplementation as probiotic yeast in PCOS; animal model determined a significant reduction in body weight and fat mass. Improved metabolic profile and TyG index as an indicator of insulin resistance, suggesting an interesting and promising role of the supplementation with *S. boulardii* in PCOS and associated metabolic complications. The ameliorative effect of *S. boulardii* on polycystic morphology further creates a thrust toward details study. It is imperative to conduct further research to focus the underlying mechanistic of *S. boulardii* in the cure of PCOS and associated health hazards

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CONFLICT OF INTEREST

Authors declares no conflicts of interest for the present article.

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PEER-REVIEWED CERTIFICATION

During the review of this manuscript, a double-blind peer-review policy has been followed. The author(s) of this manuscript received review comments from a minimum of two peer-reviewers. Author(s) submitted revised manuscript as per the comments of the assigned reviewers. On the basis of revision(s) done by the author(s) and compliance to the Reviewers' comments on the manuscript, Editor(s) has approved the revised manuscript for final publication.