

Polycystic ovarian syndrome is the most prevalent cause of infertility that is being reported in young girls hitting puberty and women of fertile age. The effect of probiotic supplementation on hormonal profile, lipid parameters, Homeostatic Model Assessment for Insulin Resistance (HOMA-IR) index, and cystic follicles were assessed to develop an alternative approach in the letrozole-induced polycystic ovarian syndrome (PCOS) model of female Wistar rats. Probiotic supplementation using *Lactiplantibacillus plantarum* ( $8.25 \times 10^{12}$ /ml), *Lactocaseibacillus rhamnosus* ( $8.40 \times 10^9$ /ml), *Enterococcus lactis* ( $8.5 \times 10^6$ /ml), and their synergist was used. For the PCOS induction letrozole (1 mg/kg) was given for 21 consecutive days orally. The PCOS induction was confirmed by measuring estrous cycle irregularity, and insulin resistance by oral glucose tolerance test (OGTT). After induction of PCOS, Diane-35 (4.5mg mg/kg), and probiotic supplementation were given for 21 days. The treatment efficacy was measured by measuring serum sex hormone profile, lipid parameters, fasting insulin level, and histopathological analysis. The one-way ANOVA (Tuckey's test) was applied to examine the data, and the p-value was less than 0.05. Overall, the probiotic supplementation individually and its synergist had very potent effects on the symptoms associated with PCOS. Androgen levels were decreased. However, only triglyceride levels were managed while other parameters like cholesterol, HDL, and LDL remained the same. The HOMA-IR parameter was also improved. Reduction in the ovarian weight and cystic follicles was also noticeable.