Efficacy of UB0316, a multi-strain probiotic formulation in patients with type 2 diabetes mellitus: A double blind, randomized, placebo controlled study

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Abstract

Background

Role of multi-strain probiotic formulations in the management of type 2 diabetes mellitus (T2DM) has rarely been reported. In the present study, the effects of the probiotic formulation, UB0316 (L. salivarius UBLS22, L. casei UBLC42, L. plantarum UBLP40, L. acidophilus UBLA34, B. breve UBB01, B. coagulans Unique IS2, 5 billion CFU each and fructo-oligosaccharides, 100 mg) in patients with T2DM were assessed.

Methods

A total of 79 eligible subjects (18–65 years, on stable metformin therapy) were randomly assigned to receive UB0316 or placebo, twice-a-day for 12 weeks. The primary endpoint was change in glycated hemoglobin (HbA1c), secondary were assessment of blood glucose levels, HOMA-IR (homeostatic model assessment of insulin resistance), insulin, body weight, and blood lipids. Quality of life, vital signs, physical investigations, safety and Physician/Subject’s Global assessment were also evaluated.

Results

Twelve week multi-strain probiotic (UB0316) supplementation significantly reduced HbA1c (7.70 ± 0.79%; p = 0.0023) and weight (67.00 ± 8.82 kg; p < 0.001) as compared to placebo (HbA1c: 8.30 ± 1.35%; weight: 67.60 ± 9.46 kg). The changes recorded in fasting blood glucose (FBG), HOMA-IR, insulin, TC, TG, HDL, and LDL levels were however not significantly altered as compared to placebo. No severe adverse events, abnormal vital and physical signs were reported. The quality of life of T2DM was significantly improved.
these authors are articulated in the ‘author contributions’.

**Competing interests:** RSM, JJA and JN are employed by Unique Biotech Limited, India, which is a manufacturer of probiotics and AT and SN are from Life Veda Treatment and Research Centre and Nanal Clinic, Mumbai, India. This does not alter our adherence to PLOS ONE policies on sharing data and materials.

**Introduction**

Type 2 diabetes mellitus (T2DM), a non-communicable metabolic disease characterized by high blood glucose levels, results from impaired insulin secretion, insulin resistance or a combination of both [1]. According to the International Diabetes Federation (IDF), in 2017, 98 million adults aged 65–79 years and 327 million adults aged 20–64 worldwide were diabetic. This number is expected to increase to 191 million (age 65–79 years) and 438 million (age 20–64) by 2045 [2]. T2DM accounts for more than 90% of all diagnosed diabetes cases and is largely the result of excess body weight and physical inactivity [1–3]. The current treatment of T2DM is costly, limited and based on management of insulin resistance by medication and lifestyle changes. There is a significant need for research on cost-effective therapies that not only control blood glucose and insulin resistance but also improve patients’ quality of life, decrease future treatment burden and serve to stabilize metabolic control [4].

Recent research on gut microbiota suggests that the bacterial community profile is a major contributing factor towards the development of T2DM, besides genetic and environmental factors [1, 5]. Significantly lower relative abundance of **Firmicutes** and higher proportions of **Bacteroidetes** and **Proteobacteria** are found in T2DM persons compared to non-diabetic counterparts [6]. The lipopolysaccharide (LPS) of enriched Gram-negative bacteria belonging to the phyla **Bacteroidetes** and **Proteobacteria** are known to trigger inflammation and play an important role in the development of T2DM [7]. In recent years, gut microbiota manipulations using probiotics is garnering a lot of interest in the control of diabetes [8]. Evidence suggests that probiotics control gut dysbiosis, improve barrier function, insulin sensitivity and reduce chronic systemic inflammations [9]. Probiotics are “live microorganisms that, when administered in adequate amounts, confer a health benefit on the host” [10]. Probiotic strains which have been implicated in regulation of sugar levels include *Lactobacillus acidophilus*, *L. casei*, *L. rhamnosus*, *L. bulgaricus*, *L. lactis*, *Bifidobacterium breve*, *B. longum*, *B. infantis*, *B. lactis*, *Streptococcus thermophilus* and *Bacillus coagulans* (*L. sporogenes*) [11].

Metformin is the first-line treatment for T2DM, known to increase glucose uptake by peripheral tissues and reduce hepatic glucose production [12]. These kind of glucose-lowering agents are known to affect the composition and diversity of gut microbiota [12, 13]. Forslund and co-workers suggest partial gut microbiota mediation during therapy with metformin [14]. In this study, we have combined multi-strains (*L. salivarius* UBLS22, *L. casei* UBLCA2, *L. plantarum* UBLP40, *L. acidophilus* UBLA34, *B. breve* UBBR01, *B. coagulans* Unique IS2, 5 billion CFU each and fructo-oligosaccharides, 100 mg), which are well documented for their in vivo clinical efficiencies to improve cholesterol profile (*B. coagulans* Unique IS2 and *L. salivarius* UBLS22) [15, 16], reduction in inflammatory markers (*L. salivarius* UBLCA2) [16], complications of liver cirrhosis (*B. coagulans* Unique IS2) [17], irritable bowel syndrome in childrens [18] and adults (*B. coagulans* Unique IS2) [19], childhood abdominal pain (*B. coagulans* Unique IS2) [20], acute diarrhoea and constipation (*B. coagulans* Unique IS2) [21, 22] and improvement in the health-related quality of life in individuals with type II diabetes mellitus (*L. salivarius* UBLS22, *L. casei* UBLCA2, *L. plantarum* UBLP40, *L. acidophilus* UBLA34, *B. breve* UBBR01, *B. coagulans* Unique IS2) [23]. The multi-strain capsule is referred as UB0316...
and in this study, the efficacy on HbA1c and blood glucose levels, HOMA-IR, weight, blood lipid profile and quality of life in T2DM adults on stable metformin therapy were assessed.

Methods

This randomized, double blind, placebo-controlled, twelve week clinical trial was reviewed and approved by Intersystem Biomedica Ethics Committee (ISBEC/NR20/KM-JV/2016) on 19th July 2016 and performed in accordance with recommendation on Good clinical practice (GCP, 2016), Declaration of Helsinki (2013) and Indian Council of Medical Research (2006). The participants were recruited and enrolled at two sites i.e. Nanal Clinic (Mumbai, India) and Life Veda Treatment and Research Centre (Mumbai, India). All the participants signed informed consent prior to their inclusion in the study. This trial was registered retrospectively with Clinical Trial Registry of India (http://ctri.nic.in) with registration number CTRI/2017/07/009164. The reason for the delay in registering at CTRI was due to some internet connectivity issues and technical hitches. The authors confirm that all ongoing and related trials for this drug/intervention are registered.

Participants

Participants of either sex, age 18 – 65 years were screened as per inclusion and exclusion criteria.

Inclusion criteria: Type 2 diabetics (on stable metformin (500 mg) monotherapy for 8 weeks prior to the screening); BMI between 23 to 32 kg/m²; glycated hemoglobin (HbA1c) between 7–9%; non pregnant females and those willing to sign informed consent form prior to participation.

Exclusion criteria: Type 1 diabetic; history of diabetic ketoacidosis; using anti-hyperglycemic medication other than metformin; fasting blood triglycerides > 400 mg/dL and/or low density lipoprotein (LDL) > 190 mg/dL; HbA1c, > 9%; known hypersensitivity to study drugs or constituents; severe systemic disease; on ayurvedic, homeopathic or herbal medicines. The participants were asked to refrain from consuming yoghurt or other similar dietary supplements during the study, no changes were made in the dietary pattern and life style of the subjects.

Discontinuation criteria: Participants were free to drop out during the study and could choose not to receive intervention; in the event of adverse reactions and laboratory abnormalities; failure to comply visit requirements as per protocol; pregnancy; use of prohibited medication; worsening of condition/disorder; frequent non-adherence to dosing regimen.

Intervention and compliance

Multi-strain probiotic UB0316 capsules (L. salivarius UBLS22, L. casei UBLC42, L. plantarum UBLP40, L. acidophilus UBLA34, B. breve UBBR01, and B. coagulans Unique IS2, 30 billion CFU and fructo-oligosaccharide, 100 mg) and placebo capsules (containing excipient malto-dextrin) were provided by Unique Biotech Limited (Hyderabad, India). Participants were instructed to take 2 capsules (UB0316 or placebo) daily after any principal meal for up to 12 weeks. During the intervention, participants were called for 3 visits at the interval of 4 weeks from baseline and assessed for schedule study outcomes. Compliance was monitored by recording the data on used (empty bottles) and unused capsules at respective study sites. All the participants were on metformin (500 mg) during the trial.

Primary outcome

Primary outcome of the present clinical investigation was the change in glycated hemoglobin (HbA1c) from baseline to week 12. HbA1c concentration (mmol/mol) in blood was evaluated
by Tina-quant® HbA1c assay using Roche cobas® 6000 modular analyzer series (Rotkreuz, Switzerland) and expressed in percent (%).

Secondary outcomes

Blood glucose. Fasting blood samples of participants were collected by standard pathological procedure and glucose levels were evaluated by GOD-POD (glucose oxidase–peroxidase) method using Roche cobas® 6000 modular analyzer series (Rotkreuz, Switzerland). The blood glucose levels (mg/dL) were estimated at baseline and week 12.

Homeostatic Model Assessment of Insulin Resistance (HOMA-IR) and Insulin. HOMA-IR was calculated based on the concentrations of fasting blood glucose and insulin. A computer-solved model was used to predict homeostatic concentrations arising from varying degrees of beta cell deficiency and insulin resistance. Serum insulin levels were measured by chemi-luminescent immunoassay (CLIA) using Roche, Elecsys 2010 Immunology analyzer (Rotkreuz, Switzerland). HOMA-IR and insulin levels were assessed at baseline and week 12.

Body weight. Participant’s body weight was measured on calibrated Omron, HBF-362, electronic scale (Omron, Karada scan, Netherlands) with an accuracy of ± 1% (kg), at baseline and week 12.

Fasting blood lipids. Fasting blood samples were collected from participants and evaluated for total cholesterol (TC: CHOD-PAP method), triglycerides (TG: GPO-PAP method), high density lipoprotein (HDL: direct enzymatic method), and low density lipoproteins (LDL: direct enzymatic method/calculated) at baseline and week 12.

Quality of life (QOL)

QOL was assessed according to the questionnaire consisting of eight domains viz. physical health, physical endurance, general health, treatment satisfaction, symptom botherness, financial worries, emotional/mental health and diet satisfaction [24], on baseline and scheduled visits.

Physician and subject global assessment

The assessment was performed on the basis of a self-administered scale which was completed by the subject or his caregiver and physician during the visits at week 4, 8 and 12. The scale of 1 indicates “worsening of condition” and 5 indicates “comprehensive relief” as to how severely activities were hindered due to diabetes.

Safety measures

Safety was measured by recording the incidence of adverse events (as per MedDRA), their severity (mild, moderate, severe) and relationship (not related, unlikely, possibly, probably, definite) to the treatment. Simultaneously, vital signs (heart rate, respiratory rate, blood pressure and temperature), physical examination and laboratory investigations (hematology) were performed at appropriate visits.

Sample size determination. SAS software (version 9.4) was used to determine sample size required to evaluate primary end point (HbA1c). Approximately, 92 participants were required for screening and 70 for evaluation of primary outcome to give 80% power to reject the null hypothesis (H0 = UB0316 – placebo = 0 verses Ha = UB0316 – placebo ≠ 0), when the true overall mean difference is minimum 1.03 with a standard deviation of 1.5 at a significance level of 0.05. The primary null hypothesis is that there is no difference between the UB0316 and the placebo with respect to the mean change from baseline in HbA1c at week 12. Rejecting this null hypothesis at a 2-sided alpha of 0.05 level will indicate the difference observed
between the UB0316 and placebo is statistically significant with respect to the mean change from baseline in Hb1Ac at week 12. In the present study, a total of 126 participants were screened, out of which 79 were enrolled and 74 completed all schedule visits.

Randomization and blinding
Randomization was performed in a 1:1 ratio by using SAS software. The generated random numbers were provided to the investigators for drug dispensing, while identity of the participants was kept blind. The probiotic and placebo capsules were provided in identical bottles to ensure double blind condition, both the capsules were identical in shape, texture and appearance, except, placebo capsules lacked the active ingredients.

Statistical analysis
Two sample t-test was performed to study the effect of probiotic treatment i.e. primary and secondary efficacy outcome measures, compared with placebo. The paired t-test was used to evaluate intragroup differences. The safety and efficacy endpoints were calculated with precision of 95% confidence interval (CI) using SAS software (Version 9.4, USA) and \( p \) value < 0.05 considered as significant.

Results
Participant flow
One-twenty-six participants were screened for eligibility criteria, out of which 79 were enrolled and randomized between January 2017 and February 2018. The randomized participants who received intended treatment [UB0316 (\( n = 40 \)) or placebo (\( n = 39 \))] and analyzed after baseline, were included in Intention to Treat (ITT) population (\( n = 79 \)). Four participants [UB0316 (\( n = 2 \)) and placebo (\( n = 2 \))] dropped out due to unavailability at scheduled visits, and 1 was a protocol deviation (UB0316 treatment) (Fig 1). The participants who completed all scheduled study visits were included in Per Protocol (PP) population [\( n = 74 \): UB0316 (\( n = 37 \)) and placebo (\( n = 37 \))]. Both PP and ITT analysis were performed and ITT analysis was considered as primary to evaluate the effect of UB0316 intervention on primary and secondary outcomes of this study. The last observation carried forward (LOCF) method was employed in ITT analysis. The baseline demographics of UB0316 and placebo treated patients were comparable (Table 1). The measured treatment compliance was 100% in both UB0316 and placebo group. All the participants were on metformin (500 mg; concomitant) medication during the trial. Rescue medicines such as human insulin and glimepride were allowed, but not utilized during the trial. This trial was performed on participants of Indian origin.

Primary outcome
Glycated hemoglobin (HbA1c) levels. By the end of treatment (12 weeks), ITT population of UB0316 treatment (7.70 ± 0.79%) showed significant (\( p = 0.0150 \)) reduction in HbA1c from the baseline (8.20 ± 0.70%), whereas placebo showed insignificant (\( p = 0.1878 \)) increase of HbA1c (8.30 ± 1.35%) as compared to the baseline (7.90 ± 0.72%) (Table 2, Fig 2). The reduction in HbA1c with UB0316 treatment was significant (\( p = 0.0023 \)), compared to placebo (Table 2). Similarly, in PP population, UB0316 supplementation significantly (\( p < 0.001 \)) reduced HbA1c as compared to placebo (Table 2).

Secondary outcomes
Blood glucose. In ITT population, UB0316 treatment by the end of 12 weeks significantly (\( p = 0.0174 \)) reduced fasting (126.30 ± 26.69 mg/dL) blood glucose levels as compared to
baseline (147.60 ± 48.40 mg/dL). (Table 2, Fig 2). Similarly, in the placebo group there was an insignificant reduction ($p = 0.7855$) in fasting sugar levels (147.80 ± 51.83 mg/dL) as compared to the baseline (150.70 ± 41.30 mg/dL), (Table 2, Fig 2). Moreover, the fasting blood glucose reduction with UB0316 treatment was insignificant ($p = 0.0709$), as compared to placebo. The analysis performed on PP population showed that UB0316 treatment significantly ($p = 0.0169$) reduce fasting blood glucose as compared with placebo (Table 2).

**Homeostatic Model Assessment of Insulin Resistance (HOMA-IR) and Insulin.** In ITT population, the HOMA-IR (2.50 ± 1.24 units) and insulin (7.70 ± 3.76 µlU/mL) levels were insignificantly ($p = 0.1412; 0.3126$) reduced from their baseline (3.00 ± 2.04 units; 8.70 ± 5.26 µlU/mL), when participants were treated with UB0316 for 12 week (Table 2, Fig 2). Similarly, placebo supplementation did not cause significant ($p = 0.6702; 0.8625$) change in either insulin (9.00 ± 4.14 µlU/mL) or HOMA IR (3.10 ± 1.38 units) levels as compared to the
baseline (8.90 ± 4.51 μlU/mL; 3.30 ± 1.86 units) (Table 2, Fig 2). The HOMA-IR and insulin reduction observed with UB0316 treatment was however non-significant ($p = 0.2944; p = 0.2084$) when compared with placebo (Table 2). The PP analysis on PP population showed similar insignificant results ($p = 0.1549; p = 0.1686$) as compared to placebo (Table 2).

**Body weight.** In ITT population, UB0316 supplementation insignificantly ($p = 0.3890$) reduced body weight (kg) from 69.20 ± 9.31 (baseline) to 67.00 ± 8.82 (week 12) (Table 2, Fig 2). Similarly, placebo showed insignificant ($p = 0.9673$) reduction from 68.00 ± 9.30 to 67.60 ± 9.46 at week 12. However, the weight reduction observed with UB0316 was significant ($p < 0.001$) compared to the placebo (Table 2). Similar results were observed in PP analysis (Table 2).

**Fasting blood lipids.** At 12 weeks, the ITT participants of UB0316 treatment showed insignificant reduction in levels of total cholesterol (TC; 167.20 ± 39.52 mg/dL), triglycerides (TG; 151.40 ± 64.10 mg/dL), and low density lipoproteins (LDL; 89.00 ± 27.37 mg/dL) from the baseline (TC: 175.10 ± 35.01; TG: 161.70 ± 74.11; LDL: 92.10 ± 34.13 mg/dL). The levels of high density lipoprotein (HDL; 52.10 ± 9.23 mg/dL) were insignificantly increased from the baseline (49.50 ± 6.30 mg/dL) (Table 2, Fig 2). In placebo, TC (174.80 ± 28.03 mg/dL) and TG (169.50 ± 86.99 mg/dL) levels were increased and HDL (48.80 ± 7.16 mg/dL) and LDL (89.40 ± 31.41 mg/dL) were decreased from their respective baseline (TC: 173.90 ± 27.81; TG: 161.70 ± 74.11; LDL: 92.10 ± 34.13 mg/dL).

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**Table 1. Baseline characteristics of participants in each group.**

| Intention to Treat | UB0316 | Placebo | Total |
|--------------------|--------|---------|-------|
| **n**              | 40     | 39      | 79    |
| Gender, n          | male/female 33/7 | 29/10  | 62/17 |
| Age, years         | mean 54.10 | 50.60  | 52.40 |
| Height, cm         | mean 161.80 | 159.90 | 160.90 |
| Weight, kg         | mean 69.20 | 68.00  | 68.60 |
| HbA1c, %           | mean ± SD 8.20 ± 0.70 | 7.90 ± 0.72 | 8.10 ± 0.71 |
| FBG, mg/dL         | mean ± SD 147.60 ± 48.40 | 150.70 ± 41.30 | 149.20 ± 44.78 |
| HOMA-IR, units     | mean ± SD 3.00 ± 2.04 | 3.30 ± 1.86 | 3.10 ± 1.95 |
| Insulin, μU/mL     | mean ± SD 8.70 ± 5.26 | 8.90 ± 4.51 | 8.80 ± 4.87 |
| TC, mg/dL          | mean ± SD 175.10 ± 35.01 | 173.90 ± 27.81 | 174.50 ± 31.47 |
| Triglycerides, mg/dL | mean ± SD 161.70 ± 74.11 | 159.60 ± 77.19 | 160.60 ± 75.17 |
| HDL, mg/dL         | mean ± SD 49.50 ± 6.30 | 49.30 ± 6.73 | 49.40 ± 6.48 |
| LDL, mg/dL         | mean ± SD 92.10 ± 34.13 | 93.60 ± 28.87 | 92.80 ± 31.45 |

**Per Protocol**

| n          | 37 | 37 | 74 |
|------------|----|----|----|
| Gender, n  | male/female 30/7 | 28/9 | 58/16 |
| Age, years | mean 53.60 | 50.50 | 52.10 |
| Height, cm | mean 161.80 | 159.90 | 160.90 |
| Weight, kg | mean 69.20 | 68.00 | 68.60 |
| HbA1c, %   | mean ± SD 8.20 ± 0.68 | 7.90 ± 0.72 | 8.00 ± 0.71 |
| FBG, mg/dL | mean ± SD 150.30 ± 49.07 | 148.90 ± 40.16 | 149.60 ± 44.53 |
| HOMA-IR, units | mean ± SD 3.10 ± 2.07 | 3.20 ± 1.86 | 3.20 ± 1.96 |
| Insulin, μU/mL | mean ± SD 9.00 ± 5.33 | 8.90 ± 4.61 | 8.90 ± 4.95 |
| TC, mg/dL   | mean ± SD 174.30 ± 35.97 | 173.30 ± 28.14 | 173.80 ± 32.08 |
| Triglycerides, mg/dL | mean ± SD 158.70 ± 68.87 | 162.50 ± 78.23 | 160.60 ± 73.22 |
| HDL, mg/dL  | mean ± SD 49.50 ± 6.37 | 49.40 ± 6.72 | 49.50 ± 6.50 |
| LDL, mg/dL  | mean ± SD 90.00 ± 34.50 | 92.40 ± 29.05 | 91.20 ± 31.69 |

HbA1c: glycated hemoglobin; FBG: Fasting blood glucose; HOMA-IR: homeostatic model assessment of insulin resistance; TC: total cholesterol; HDL: high density lipoproteins; LDL: low density lipoproteins.
Table 2. Primary and secondary outcomes (baseline to week 12) of the Intention-to-Treat and Per Protocol populations.

|                          | UB0316 (mean ± SD) | Placebo (mean ± SD) | Absolute change from baseline to visit (mean ± SD, 95% CI) | p value^ | p value§ |
|--------------------------|--------------------|---------------------|-----------------------------------------------------------|----------|----------|
|                          | Baseline n = 40    | Week 12 n = 40      | Baseline n = 39                                          |          |          |
| HbA1c, %                 | 8.20 ± 0.70        | 7.70 ± 0.79         | 7.90 ± 0.72                                              | 8.30 ± 1.35 | 0.00 ± 1.09 (−0.19, −0.29) | 0.0023 | 0.0150 | 0.1878 |
| FBG, mg/dL               | 147.60 ± 48.40     | 126.30 ± 26.69      | 150.70 ± 41.30                                           | 147.80 ± 51.83 | 12.30 ± 45.51 (2.06, 22.45) | 0.0709 | 0.0174 | 0.7855 |
| HOMA-IR, units           | 3.00 ± 2.04        | 2.50 ± 1.24         | 3.30 ± 1.86                                              | 3.10 ± 1.38 | 0.40 ± 1.70 (−0.02, 0.74) | 0.2944 | 0.1412 | 0.6702 |
| Insulin, μU/mL           | 8.70 ± 5.26        | 7.70 ± 3.76         | 8.90 ± 4.51                                              | 9.00 ± 4.14 | 0.40 ± 4.25 (−0.51, 1.40) | 0.2084 | 0.3126 | 0.8625 |
| Weight, kg               | 69.20 ± 9.31       | 67.00 ± 8.82        | 68.00 ± 9.30                                             | 67.60 ± 9.46 | 1.00 ± 1.77 (0.55, 1.37) | < 0.001 | 0.3890 | 0.9673 |
| TC, mg/dL                | 175.10 ± 35.01     | 167.20 ± 39.52      | 173.90 ± 27.81                                           | 174.80 ± 28.03 | 3.60 ± 34.83 (−4.24, 11.36) | 0.2601 | 0.3452 | 0.8855 |
| TG, mg/dL                | 161.70 ± 74.11     | 151.40 ± 64.10      | 159.60 ± 77.19                                           | 169.50 ± 86.99 | 0.30 ± 73.52 (−16.20, 16.74) | 0.2246 | 0.5108 | 0.5946 |
| HDL, mg/dL               | 49.50 ± 6.30       | 52.10 ± 9.23        | 49.30 ± 6.73                                             | 48.80 ± 7.16 | −1.10 ± 9.50 (−3.23, 1.02) | 0.1541 | 0.1440 | 0.7794 |
| LDL, mg/dL               | 92.10 ± 34.13      | 89.00 ± 27.37       | 93.60 ± 28.87                                            | 89.40 ± 31.41 | 3.70 ± 30.64 (−3.21, 10.52) | 0.8718 | 0.6552 | 0.5381 |
| Absolute change from baseline to visit (mean ± SD, 95% CI) | | | | | |

- HbA1c: glycated hemoglobin; FBG: fasting blood glucose; HOMA-IR: homeostatic model assessment of insulin resistance; TC: total cholesterol; TG: triglycerides; HDL: high density lipoproteins; LDL: low density lipoproteins.
- #: intergroup (two sample t test)
- §: intragroup (paired t test).

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159.60 ± 77.19; HDL: 49.30 ± 6.73; LDL: 93.60 ± 28.87 mg/dL) (Table 2, Fig 2). The changes (intragroup) were non-significant. Overall, UB0316 treatment did not significantly improve fasting blood lipid profile (TC: p = 0.2601; TG: p = 0.2246; HDL: p = 0.1541; LDL: p = 0.8718) compared to placebo (Table 2). Similarly, in PP analysis, UB0316 supplementation failed to significantly improve fasting blood lipid profile (TC: p = 0.3088; TG: p = 0.2331; HDL: p = 0.1105; LDL p = 0.8151), as compared to placebo (Table 2).

Quality of life (QOL)

The total score of QOL of ITT population was significantly improved with 12 weeks of UB0316 treatment (p = 0.0130), compared to placebo. Among the eight domains evaluated, five of the domains namely physical health (p = 0.0401), physical endurance (p = 0.0242), general health (p = 0.0062), treatment satisfaction (p = 0.0493), and diet satisfaction (p = 0.0352) improved significantly as compared with placebo. There was no significant change in the other three domains viz. symptom botherness (p = 0.6159), emotional/mental health (p = 0.0909) and financial worries (p = 0.3047) as compared to placebo (Table 3, Fig 3). However, symptom botherness and emotional/mental health were significantly (p < 0.001) improved from their baseline in UB0316 group (Table 3). The PP analysis, the total score of QOL was significant improved with UB0316 treatment (p = 0.0057) as compared to placebo (Table 3). The results of effect of treatment and placebo on QOL at all schedule visits are shown in S1 and S2 Tables.
Physician and subject global assessment

Physician global assessment for UB0316 treatment on ITT and PP population between week 4 to 12 showed non-significant changes ($p = 0.0780; p = 0.0769$), compared to placebo (S3 Table). However, the changes observed in UB0316 group from the baseline were significant in both ITT and PP analysis ($p < 0.001$). The subject global assessment for UB0316 treatment on both ITT and PP population was significant ($p = 0.0166; p = 0.0163$) as compared to placebo (S4 Table). Similarly, the changes observed in UB0316 group from the baseline were significant in both ITT and PP analysis ($p < 0.001$).

In physician assessment on 12 week UB0316 supplementation, 2.50% participants of ITT population had complete relief, 55.00% considerable relief, 37.50% remained unchanged, 2.50% somewhat relieved and 2.50% experienced worse conditions. However, with placebo, 23.08% participants' experienced considerable relief, 64.10% remained unchanged, 7.69% somewhat relieved and 5.13% worse (S5 Table). The assessment on PP population showed 59.46% participants had considerable relief, 35.14% considerable relief, 35.14% remained unchanged, 2.70% somewhat relieved and 2.70% worse with UB0316 treatment, while 24.32% had considerable relief, 62.16% remained unchanged, 8.11% somewhat relieved and 8.11% experienced worse conditions (S5 Table).

In subject assessment (ITT population), 12 week UB0316 treatment provided complete relief to 2.50% participants while 52.50% had considerable relief, 40.00% remained unchanged, 2.50% somewhat relieved and 2.50% experienced worse conditions, whereas 23.08% participants showed considerable relief, 61.53% remained unchanged, 7.69% somewhat relieved and 7.69% experienced worse conditions with placebo supplementation (S6 Table). In PP population, 2.70% participants with UB0316 showed complete relief, 56.76% considerable relief, 35.14% remained unchanged, 2.70% somewhat relieved and 2.70% worse, whereas 24.32% participants with placebo had considerable relief, 59.46% remained unchanged, 8.11% somewhat relieved and 8.11% experienced worse conditions (S6 Table).
Table 3. Quality of life assessment of the Intention-to-Treat and Per Protocol populations.

|                          | UB0316 (mean ± SD) | Placebo (mean ± SD) | Absolute change from baseline to visit (mean ± SD, 95% CI) | p value<sup>a</sup> | p value<sup>b</sup> |
|--------------------------|--------------------|---------------------|--------------------------------------------------------------|---------------------|---------------------|
|                          | Baseline n = 40    | Week 12 n = 40      | Baseline n = 39                                             | Week 12 n = 39      |                      |
| **Intention to Treat**   |                    |                     |                                                              |                     |                      |
| Physical health          | 14.20 ± 5.33       | 15.40 ± 4.43        | 18.00 ± 3.87                                                | −3.50 ± 3.61 (−4.29, −2.67) | 0.0401              |
| Physical endurance       | 14.30 ± 5.74       | 14.70 ± 5.17        | 16.90 ± 4.23                                                | −3.00 ± 3.07 (−3.70, −2.33) | 0.0242              |
| General health           | 6.50 ± 2.08        | 6.50 ± 1.64         | 7.30 ± 1.59                                                 | −1.50 ± 2.19 (−1.98, −1.00) | 0.0062              |
| Treatment satisfaction   | 9.90 ± 2.75        | 10.40 ± 3.00        | 11.70 ± 2.33                                                | −1.80 ± 2.14 (−2.24, −1.28) | 0.0493              |
| Symptom botherness       | 8.80 ± 2.17        | 8.70 ± 1.98         | 10.40 ± 2.23                                                | −1.80 ± 2.05 (−2.34, −1.33) | 0.6159              |
| Emotional/mental health  | 12.10 ± 4.98       | 13.40 ± 4.87        | 15.60 ± 4.06                                                | −2.70 ± 3.01 (−3.38, −2.03) | 0.0909              |
| Diet satisfaction        | 6.80 ± 2.24        | 6.90 ± 2.42         | 7.70 ± 2.25                                                 | −1.20 ± 1.76 (−1.64, −0.85) | 0.0352              |
| Financial worries        | 12.90 ± 2.75       | 12.50 ± 2.23        | 13.20 ± 2.32                                                | −0.90 ± 1.76 (−1.27, −0.48) | 0.3047              |
| Total score              | 85.50 ± 23.53      | 88.40 ± 20.30       | 100.70 ± 15.96                                              | −16.40 ± 14.62 (−19.63, −13.08) | 0.0130              |
| **Per Protocol**         |                    |                     |                                                              |                     |                      |
| Physical health          | 14.00 ± 5.12       | 15.10 ± 4.34        | 17.80 ± 3.83                                                | −3.70 ± 3.65 (−4.51, −2.82) | 0.0227              |
| Physical endurance       | 14.10 ± 5.53       | 14.40 ± 5.06        | 16.60 ± 4.11                                                | −3.20 ± 3.10 (−3.88, −2.44) | 0.0097              |
| General health           | 6.50 ± 2.10        | 6.40 ± 1.60         | 7.30 ± 1.63                                                 | −1.60 ± 2.20 (−2.12, −1.10) | 0.0062              |
| Treatment satisfaction   | 9.80 ± 2.77        | 10.40 ± 3.04        | 11.70 ± 2.32                                                | −1.90 ± 2.17 (−2.37, −1.36) | 0.0309              |
| Symptom botherness       | 8.70 ± 2.19        | 8.80 ± 2.02         | 10.50 ± 2.22                                                | −1.90 ± 2.07 (−2.37, −1.41) | 0.5044              |
| Emotional/mental health  | 11.90 ± 4.91       | 13.10 ± 4.77        | 15.40 ± 4.08                                                | −2.90 ± 3.01 (−3.60, −2.21) | 0.0694              |
| Diet satisfaction        | 6.60 ± 2.15        | 6.80 ± 2.39         | 7.60 ± 2.24                                                 | −1.30 ± 1.80 (−1.73, −0.89) | 0.0230              |
| Financial worries        | 12.80 ± 2.66       | 12.40 ± 2.28        | 13.10 ± 2.38                                                | −0.90 ± 1.80 (−1.35, −0.52) | 0.2754              |
| Total score              | 84.40 ± 22.80      | 87.20 ± 19.97       | 100.00 ± 15.94                                              | −17.30 ± 14.58 (−20.72, −13.96) | 0.0057              |

<sup>a</sup>: intragroup (paired t test)
<sup>b</sup>: intergroup (two sample t test)

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Safety measures

During the twelve week UB0316 treatment, 2 participants (PP population) showed either constipation and or flatulence. According to MedDRA System Organ Class, these events are classified into gastrointestinal system organ class with preferred term of constipation and flatulence. Furthermore, the events were rated as mild (flatulence) and moderate (constipation), not related and assessed as unlikely relation to the treatment. Placebo had no adverse events to report. Hematology, vital signs and physical examinations remains normal and within normal range throughout the trial period (<sup>57</sup>, <sup>58</sup> and <sup>59</sup> Tables). No other severe adverse events and deaths were reported in this study.

Discussion

In this study, we have shown that the daily (twice a day) administration of UB0316 to T2DM patients (with HbA1c between 7–9% and on stable metformin: 500 mg) therapy for 12 weeks significantly improved HbA1c (primary outcome) along with a concomitant decrease in weight as compared to placebo. FBG, HOMA-IR, insulin, TC, TG, HDL, and LDL were however not significant as compared to placebo. Overall, UB0316 significantly improved quality of life of T2DM patients.
The primary and secondary outcomes of the trial were evaluated for both ITT and PP population to avoid bias prone conclusions due to protocol deviations and dropouts [25]. The ITT analysis was considered as primary and at around 93.67% of ITT population was used to perform PP analysis.

HbA1c is an indicator of chronic glycaemia, which gives integrated glycemic index over the entire lifespan (120 day) of red blood cells [26]. Evidences suggest that probiotics control chronic glycaemia by stabilizing gut dysbiosis, improve barrier function, insulin sensitivity and reducing chronic systemic inflammations [9]. A recent clinical study conducted using a multi-strain probiotic formulation showed significant reduction in HbA1c after 8 weeks of treatment [27]. However, the trial was conducted on small population and there was no information on use of metformin which is considered as the first line of treatment. In the present 12 week clinical investigation, we have shown that multi-strain probiotic UB0316 as an adjuvant to metformin significantly reduced HbA1c. The change noted for placebo from the baseline was non-significant, which is in accordance to the results of Mobini et al. [28], that participants of placebo (most of them on metformin) showed no significant change in HbA1c for 12 weeks. Moreover, the 12 week trial with single strain probiotics did not lead to reduction in HbA1c in T2DM participants (on metformin). On contrary, 6 or 8 weeks studies using fermented products with two or more probiotic strains reported reductions in HbA1c, but there is no data available for metformin use during the trial [29–31]. Overall, the results suggested that multi-strain probiotics are much effective in reduction of HbA1c.

FBG levels estimated in ITT population of this study were non-significant may be due to LOCF. On the contrary, at 12 weeks, FBG levels in PP population were significantly ($p = 0.0169$) reduced with UB0316 treatment as compared with placebo. The FBG reduction is
in accordance with recent meta-analysis that probiotic supplementation has significant FBG lowering effects when given along with antidiabetic drug in diabetic individuals [32].

Unbalanced blood lipid profile is one of the main symptoms of T2DM, which is known to intensify risk factors associated with cardiovascular diseases [33]. The participants recruited in this study had lipid profile values close to normal which could be due to the lipid balancing effects of metformin monotherapy [34]. Lipid profile changes in the UB0316 treatment group were insignificant as compared with placebo suggesting negligible effects of probiotics + metformin on lipid profile. However, in previous studies we have shown that daily supplementation of *B. coagulans* Unique IS2 for 60 days and *L. salivarius* UBLS-22 for 42 days significantly improve lipid profile of hypercholesterolemic [15] and or healthy individuals [16]. Moreover, the results of present investigation warrant need of clinical studies with large population, duration, customized diet and life style to come to a firm conclusion.

A recent, 12 week double blind, randomised, multi-strain probiotic trial conducted on T2DM patients showed that probiotic (*B. bifidum* W23, *B. lactis* W52, *L. acidophilus* W37, *L. brevis* W63, *L. casei* W56, *L. salivarius* W24, *Lactococcus lactis* W19 and W58, 2.5 × 10⁹ CFU/g; 2 g sachets) supplementation for 12 week significantly reduced HOMA-IR in medication naïve T2DM patients. The levels of insulin changed but remained insignificant as compared to placebo [35]. In the present study, we report multi-strain probiotic UB0316 supplementation for 12 weeks as an adjuvant to metformin reduced both HOMA-IR and insulin levels as compared to placebo in T2DM participants. Though, the changes were not significant, they appear important in maintaining a stable equilibrium between the conjuncted elements of T2DM, which are vital in the prevention and management of the diabetes.

Counteracting excessive weight gain is important to prevent T2DM [27]. However, there is no clear evidence that in patients with T2DM weight loss can reduce microvascular or cardiovascular complications [36, 37]. In this study, 12 week treatment of UB0316 significantly reduced weight of T2DM patients as compared with placebo, which is in agreement with the several previous findings [38]. However, the insignificant weight reduction observed in intragroup analysis warrant need of clinical studies with large population, duration, customized diet and life style.

T2DM has an impact on quality of life and socializing which may in turn may have negative impacts on mental health and long term T2DM management [39]. In the present investigation, we observed improvement in QOL of both probiotic and placebo treated groups with metformin, however, QOL scores were significantly higher in probiotic as compared to placebo. These results suggested that probiotic as an adjuvant to metformin improved QOL limitations associated with T2DM. Recently, Venkataraman et al. [23] showed that 12 week UB0316 capsule supplementation improved health-related quality of life in individuals with type II diabetes mellitus. Moreover, the improvement in scores of subject as well as physician global assessment suggested good tolerability profile of UB0316. There are however a few limitations to this study: Though sufficiently powered, the total number of subjects was not large; additionally the study duration was for only 12 weeks; no changes were made in the dietary pattern and life style of the subjects, which could be one of the reasons that no clear-cut significant differences in most of the secondary outcome measures between probiotic and placebo treatments were evident. Besides this, gut microbiota changes were not evaluated for the participants of both the groups.

**Conclusions**

In conclusion, consumption of UB0316 along with metformin for a period of 12 weeks significantly lowered HbA1c and weight as compared to placebo with metformin. There was a trend
towards improvement of FBG, HOMA-IR, insulin and lipid levels though it was not significant in T2DM patients. To conclude, UB0316 is safe and well-tolerated probiotic multi-strain formulation that can be given along with metformin which is used as the first line of treatment in the management of T2DM.

**Supporting information**

S1 Table. ITT analysis of change in Quality of Life (QOL) as compared from baseline.

S2 Table. PP analysis of change in Quality of Life (QOL) as compared from baseline.

S3 Table. Change from visit 1 to the end of visit 2 and visit 3 in physician global assessment of T2DM.

S4 Table. Change from visit 1 to the end of visit 2 and visit 3 in subject’s global assessment of T2DM.

S5 Table. Summary of physician global assessment of T2DM.

S6 Table. Summary of subject’s global assessment of T2DM.

S7 Table. Summary of hematology of T2DM participants.

S8 Table. Summary of vital signs of T2DM participants.

S9 Table. Summary of physical examination of T2DM participants.

S10 Table. CONSORT 2010 Checklist.

S1 Text. Diabetes study protocol.

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