Efficacy and safety of berberine hydrochloride for glycemic control in prediabetic individuals: a double-blind, placebo-controlled, and randomized trial

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Abstract

Background: Prediabetes and diabetes involve alterations in glucose homeostasis, including elevated fasting blood glucose and impaired glucose tolerance. Berberine has been identified as a potential regulator of glucose homeostasis with implications on the management of type 2 diabetes mellitus (DM). Given a paucity of data on berberine in prediabetes, evaluation of its effect in prediabetic individuals may prove clinically valuable. The present study aimed to investigate the effect of daily oral berberine on markers of glycemic control and insulin resistance among prediabetic individuals.

Methods: A randomized, double-blinded, placebo-controlled trial was conducted for 12 weeks among 34 prediabetic individuals as defined by the American Diabetes Association (fasting plasma glucose (FPG) between 5.6 and 6.9 mmol/L, glycosylated hemoglobin (HbA_{1c}) between 5.7% and 6.4%, or 2-hour 75-gram oral glucose tolerance test (2h-OGTT) between 7.8 and 11.1 mmol/L). HIMABERB® 500 mg was given three times daily to the treatment group, and placebo was administered three times daily to the control group. Glycemic control markers and physical parameters were evaluated for both groups on days 0, 28, 56, and 84. The glycemic control markers assessed included FPG, fasting insulin (FI), 2h-OGTT, HbA_{1c}, and homeostatic model assessment-insulin resistance (HOMA-IR). The observed outcomes were analyzed using independent t-test statistics to determine the significance of differences over time after treatment initiation and between treatment and control groups.

Results: Significant decreases in all markers of glycemic control were observed in the treatment group at intermediate time points and the endpoint of the study compared to baseline levels and to the control group. For the treatment group, FPG decreased from 6.75 ± 0.23 mmol/L to 5.33 ± 0.28 mmol/L, FI from 9.81 ± 0.36 to 7.88 ± 0.52 mmol/L, 2h-OGTT from 10.44 ± 0.52 to 8.12 ± 0.40 mmol/L, HbA_{1c} from 6.40% ± 0.20% to 5.43% ± 0.21%, and HOMA-IR from 3.61 ± 0.31 to 2.41 ± 0.14. The decreases in glycemic control markers compared to the control group were clinically and statistically significant ($p<0.00001$). No severe adverse effects, kidney or liver toxicity were detected.

Conclusion: After 12 weeks, berberine (HIMABERB®) intervention in prediabetic individuals significantly reduced glycemic control markers, with mean FPG and 2h-OTGG being reduced to below prediabetic thresholds, supporting the investigation of the use of HIMABERB® for delaying progression to diabetes mellitus.

Trial registration: http://ctri.nic.in (CTRI/2021/12/038751) 20/12/2021

1. Background

Diabetes continues to be a major global health concern with a rapidly rising incidence. The incidence of conversion of prediabetes to diabetes has increased greatly. According to the International Diabetes Federation (IDF), 382 million people globally (8.3% of adults) are affected by diabetes, and the incidence is predicted to increase beyond 592 million in the next 25 years[1]. In a prediabetic individual, the blood

sugar level is higher than normal but not high enough to be diagnosed with diabetes. In the U.S., 35-38% of people are affected by prediabetes[1], [2]. According to the American Diabetes Association (ADA), an individual can be considered prediabetic when FPG is 5.6-6.9 mmol/L, glycated hemoglobin (HbA_{1c}) is 5.7%-6.4%, or postprandial glucose is 140-199 mg/dl[3]. Although prediabetes does not display any physical symptoms, it nearly always precedes type 2 diabetes mellitus, which further increases the risk of microvascular diseases such as retinopathy, nephropathy, and neuropathy [4], [5].

There is a lack of robust recommendations to prevent or delay the progression of prediabetes to type 2 diabetes because of limited pharmacological options and scarce literature on nutraceutical therapy with favorable safety profiles. ADA recommends lifestyle behavior changes to prevent the progression of prediabetes to diabetes, including weight loss, moderate-intensity physical exercise, dietary changes, and metformin pharmacotherapy[6]. Cinnamon[7], fenugreek[8], nanocurcumin[9], tulsi[10], and soybean extract [11] have been to manage glucose metabolism. The identification of a safe, durable, and cost-effective adjunct to effectively and consistently reduce the progression from prediabetes to type 2 diabetes mellitus remains an unmet goal.

Berberine is a naturally found alkaloid with a quaternary-based chemical structure that is known to have a hypoglycemic effect. It has been used in Ayurveda in India, traditional Chinese medicine, and Middle Eastern countries for over 400 years[12]. It has recently been reported to effectively lower blood glucose and lipid levels[13][14]. In the preventive era, efforts are increasingly focused on preventing or delaying the transition from prediabetes to diabetes mellitus[15], [16]. However, most studies on berberine have focused on type 2 diabetes mellitus patients only, with limited studies on prediabetic individuals. The present double-blinded, randomized, and placebo-controlled trial was designed to evaluate the efficacy of HIMABERB® Berberine on glycemic control markers in otherwise healthy prediabetic individuals.

2. METHODS

2. 1. Preparation of HIMABERB® and placebo

HIMABERB® (a water-extracted berberine hydrochloride) was supplied by Gramen Botanicals Pvt. Ltd, India. In brief, *Berberis aristata* aqueous root extract was prepared by maceration at room temperature via water extraction. The extract was then concentrated and dried to achieve a purity of at least 97% berberine hydrochloride, and capsules were made with 500 mg of HIMABERB® in each capsule. Microcrystalline cellulose powder was chosen as the placebo due to its inert nature and inability to impact blood glucose markers. Opaque capsules were used for both HIMABERB® and placebo to mask the color difference and maintain the double-blind study design.

2. 2. Ethical Approval:

This study (approval no. SOA/IDS/IRB/2021-10) was approved by the IEC, IMS & SUM Hospital, Siksha ’O’ Anusandhan University, K-8 Kalinga Nagar, Bhubaneswar, Odisha-75003 on May 17, 2021. The study

protocol was prospectively registered at http://ctri.nic.in (CTRI/2021/12/038751) on December 20, 2021.

2.3. Study Design:

The study was a randomized, double-blinded (to study personnel, those analyzing data and patients), and placebo-controlled clinical trial to evaluate the efficacy of HIMABERB® in prediabetic individuals for 12 weeks. *It was hypothesized that HIMABERB® Berberine should improve the markers of glycemic control more effectively than the placebo.* The study began on January 7, 2021. Screening of participants was conducted in April and May of 2021 with the first recruitment on April 13, 2021 and randomization in June of 2021 and follow-up for 84 days. The study began After receiving a detailed explanation and providing written informed consent, 34 participants at IMS & SUM Hospital, Siksha 'O' Anusandhan University were assigned to the treatment and control groups by block randomization with parallel assignment to ensure bias reduction. Seventeen participants were assigned to each group. HIMABERB® 500 mg was given three times daily to the treatment group, and placebo was administered three times daily to the control group for 84 days. Dosing was as per the manufacturer's instructions and after consultation with an endocrinologist. This dosing regimen is supported by previously published literature on berberine, with typical dosing of 0.5 to 1.5 g/day in trials treating diabetes mellitus[17]. Each patient kept a supplement and diet diary and a record of any adverse events on an SAE (Severe Adverse Effects) form as part of the diary.

Figure 1 shows the flow chart of participants through each stage of the clinical study. The participants were screened for the clinical study based on the inclusion and exclusion criteria as detailed in **Table 1**, and candidates who met any exclusion criteria were eliminated. The screened participants who met all prediabetes criteria as defined by the American Diabetes Association (FPG between 5.6 and 6.9 mmol/L; HbA_{1c} between 5.7% and 6.4%; and 2-hour 75-gram oral glucose tolerance test (2h-OGTT) between 7.8 and 11.1 mmol/L) were enrolled in the study[3]. A total of 34 individuals were enrolled, and the study was conducted over 12 consecutive weeks (84 days). Patients were randomized using computer generated sequence and block randomization with 1:1 block size into control and test groups (n=17 each). Patients and study personnel were blinded to the allocation of each patient by random allocation and sealed correspondence. Random allocation and enrollment were performed by the principal investigator. The first follow-up was scheduled on Day 28, the second follow-up on Day 56, and the third and final follow-up was scheduled for Day 84. All participants visited the study site, located at Siksha O Anusandhan (Deemed to be University), Bhubaneshwar, Odisha, 751030, India, at baseline and at the end on the 84^{th} day. The other two follow-up visits on Days 28 and 56 were conducted at each participant's home. At every visit, the supplement and diet diary and SAE forms were evaluated and verified. Follow up for 84 days was chosen to allow reliable evaluation of glycemic control using HbA1c over an approximately 3-month period.

Table 1

Inclusion and exclusion criteria for the study

INCLUSION CRITERIA
1. Age 18 to 55 years 2. Male or female gender 3. Females of childbearing age to agree to use birth control methods during the trial period and show a negative pregnancy test at the time of recruitment 4. Fulfillment of the prediabetic diagnostic criteria as per the American Diabetes Association, taken within 12 weeks. (FPG between 5.6 and 6.9 mmol/L; HbA_{1c} between 5.7% and 6.4%; and 2h-OGTT between 7.8 and 11.1 mmol/L) 5. Agreement to continue the current diet and refrain from any new supplements 6. Ability to read and understand English 7. Agreement to provide informed consent to the trial with the ability to understand the risks and benefits of the protocol 8. Willingness to complete intake form, supplement and diet diary, and records associated with the study 9. Willingness to cooperate for follow-up calls and visits
EXCLUSION CRITERIA
1. Pregnant or lactating women, or women planning to become pregnant in the next 12 weeks 2. Transgender individuals or individuals taking a hormonal injection 3. Comorbidities including malnourishment, impaired hepatic or renal function, cardiac diseases, any acute/severe diseases, or having undergone bariatric surgery 4. Currently taking weight loss medications, any oral hypoglycemic medication, insulin injection, or steroids 5. Current daily use of herbal/non-herbal supplements 6. Daily tobacco use, or individuals with any substance abuse issues in the past or present 7. Known allergies to any substances in the product 8. Any significant neurological and/or psychiatric conditions

2.4. Observation indicators:

Preliminary screening and assessment were carried out for every individual to collect medical history, physical examination results, blood pressure, heart rate, body weight, waistline, liver function, kidney function, FPG, FI, 2h-OGTT, HbA_{1c}, and HOMA-IR. Participants fasted for at least 8 hours before blood samples were collected at approximately 0800 h, and samples were tested using an automatic biochemical analyzer (Cobas Integra 400 Roche, make - 2018, Germany). All parameters were recorded at every follow-up visit. HOMA-IR was calculated by Equation (1):

$$HOMA - IR = \frac{(FIxFPG)}{22.5} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots Equation(1)$$

2.5. Safety assessment:

Participants were interviewed regarding side effects and adverse reactions during the follow-up visits. SAE forms were part of the patient diary, which were explained and reinforced at every follow up visit and on telephone calls once a week. The supplement and diet diary were checked at each follow-up to ensure completion and uniformity. To evaluate liver and kidney function and ensure safety for individuals in the treatment group, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), and blood urea nitrogen to serum creatinine ratio (SC) levels were measured at the start of the study, 28 days, and 84 days. As the follow-up duration was limited to 84 days, SC ratios were used to quantifiably assess short-term renal toxicity for statistical analysis. Participants were routinely reminded to maintain the supplementation regimen and to update the supplement and diet diary regularly.

2.6. Statistical methods:

Data collected on the randomized participants were tabulated as per the guidelines of CONSORT (Consolidated Standards of Reporting Trials). The statistical analysis was performed using GraphPad Prism (Version 9.3.1, GraphPad Software San Diego, CA 92108) software for Windows computers. Descriptive analysis and independent t-tests were performed for the obtained tabulated values with statistical significance set at $p < 0.05$. Data are presented as the mean ± SD. Sample size was determined to achieve statistical power of 80% and a significance level of 0.05 using two-sided t –test, two groups and equal variance. Anticipating a difference in means of 0.91 SD and 10% attrition, a total sample size of 34 was derived.

3. Results

3.1. Participant characteristics: A total of 34 prediabetic individuals fulfilling the inclusion and exclusion criteria were enrolled (n=17 in each group). Baseline features were matched for the treatment and control groups (**Table 2)**. Adherence to the supplement regimen was found to be equivalent in the treatment and control groups.

Table 2

Baseline demographic characteristics (mean ± SD)

Variables	Treatment Group (n=17)	Control Group (n=17)
Age (years)	53.5 ± 8.9	54.9 ± 8.6
Male: Female	1.4:1	1.1:1
Weight (kg)	78.7 ± 19.1	80.0 ± 16.4
Waistline (cm)	85.8 ± 7.9	85.9 ± 8.9
Hipline (cm)	93.6 ± 9.5	92.8 ± 9.5
BMI ($^{kg/m2}$)	25.6 ± 3.3	25.4 ± 5.0

BMI: Body Mass Index

3.2. Efficacy:

A detailed analysis of the treatment group at different time points revealed that the glucose marker values consistently declined over time (**Figure 2**).

Table 3 summarizes the investigated endpoints for glycemic control parameters (FPG, FI, 2h-OGTT, HbA_{1c}, and HOMA-IR profiles) at baseline (t_0), after 28 days (t_1), after 56 days (t_2), and after 84 days (t_3) of HIMABERB® supplementation or placebo, with the relative values compared to the baseline value (t_0) with t_0 set at (1). All measured glycemic control markers showed a significant reduction over time in the treatment group. From t_0 to t_3, in 84 days, FPG values saw a total 21.01% reduction in mean values, FI saw 19.68% reduction in mean values, 2h-OGTT mean values were down by 22.15, HbA_{1c} mean values were down by 15.17% and HOMA-IR units declined by 33.39% in mean values (p<0.00001). There was also a consistent decline in all parameters from baseline (t_0), to 28 days (t_1), 56 days (t_2), and 84 days (t_3) as shown in **Table 3**. No measures were significantly decreased in the placebo group at any time point.

Table 3

Effect of treatment on FPG, FI, 2h-OGTT, HbA_{1c}, and HOMA-IR profiles relative to baseline (t_0), after 28 days (t_1), 56 days (t_2), and 84 days (t_3) of HIMABERB® or placebo control treatment.

	Parameters	t_0 (0 Day) ± SD	t_1 (28 Days) ± SD	t_2 (56 Days) ± SD	t_3 (84 Days) ± SD
Treatment Group	FPG (mmol/L)	1.00 ± .03	0.91 ± .03*	0.81 ± .04*	0.79 ± .03*
	FI (μIU/mL)	1.00 ± .04	0.92 ± .02*	0.83 ± .03*	0.80 ± .05*
	2h-OGTT (mmol/L)	1.00 ± .05	0.92 ± .04*	0.82 ± .06*	0.78 ± .07*
	HbA_{1c}(%)	1.00 ± .04	0.94 ± .03*	0.86 ± .04*	0.85 ± .05*
	HOMA-IR (units)	1.00 ± .09	0.84 ± .08*	0.75 ± .10*	0.68 ± .10*
Control Group	FPG (mmol/L)	1.00 ± .07	0.99 ± .01	0.98 ± .01	0.98 ± .02
	FI (μIU/mL)	1.00 ± .03	0.99 ± .02	0.99 ± .02	0.99 ± .04
	2h-OGTT (mmol/L)	1.00 ± .06	0.99 ± .01	0.99 ± .01	1.00 ± .06
	HbA_{1c}(%)	1.00 ± .04	0.99 ± .01	0.99 ± .02	0.99 ± .04
	HOMA-IR (units)	1.00 ± .08	0.97 ± .03	0.95 ± .03	0.99 ± .08

*p-values relative to t_0 <0.00001. Differences relative to t_0 were non-significant in the control group.

FPG after 84 days was significantly lower in the treatment group than in the control group, with a mean FPG of 5.33 ± 0.28 mmol/L for the treatment group and 6.16 ± 0.44 mmol/L for the control group (p<0.00001). FI after 84 days was also significantly lower in the treated-group compared to controlled-group, with a mean FI of 7.88 ± 0.52 μIU/mL for the treatment group and 9.76 ± 0.37 μIU/mL for the control group (p<0.00001). Similar significant reductions were seen after 84 days in 2h-OGTT, with a mean value in the treatment group of 8.12 ± 0.40 mmol/L which was significantly lower than the control group with a mean value of 9.68 ± 0.51 mmol/L (p<0.00001). HbA_{1c} values after 84 days were also significantly lower for the treatment group (5.43 ± 0.21 %) in comparison with the control group (6.10 ± 0.24%) (p<0.00001). HOMA-IR showed a similar significant reduction for the treated-group with mean values after 84 days at 2.41 ± 0.14 units in comparison with control group (3.40 ± 0.28 %) (p<0.00001), as shown in **Table 4**. The treated group showed statistically significant reductions in these values at all three time points, 28, 56, and 84 days, compared to baseline values, and all glycemic measures were found to be significantly lower in the treated-group in comparison controlled-group (p<0.00001).

Table 4

Changes in FPG, FI, 2h-OGTT, $HbA_{1c,}$ and HOMA-IR after intervention (at Day 84) in the treatment and control groups, mean ± SD (Significance level p value < 0.00001)

Parameters	Treatment Group (n=17)	Treatment Group (n=17)	Control Group (n=17)	Control Group (n=17)	*p* value Treatment vs Control
	Day 0	Day 84	Day 0	Day 84	Day 84
FPG (mmol/L)	6.75 ± 0.23	5.33 ± 0.28*¥	6.31 ± 0.44	6.16 ± 0.44	*p<0.00001*
FI (µIU/mL)	9.81 ± 0.36	7.88 ± 0.52*	9.85 ± 0.35	9.76 ± 0.37	*p<0.00001*
2h-OGTT (mmol/L)	10.44 ± 0.52	8.12 ± 0.40*	9.71 ± 0.63	9.68 ± 0.51	*p<0.00001*
$HbA1_c$ (%)	6.40 ± 0.20	5.43 ± 0.21*¥	6.15 ± 0.26	6.10 ± 0.24	*p<0.00001*
HOMA-IR (units)	3.61 ± 0.31	2.41 ± 0.14*	3.45 ± 0.26	3.40 ± 0.28	*p<0.00001*

All values represent means. *p-values for differences between day 84 and day 0 <0.00001. ¥ below prediabetic threshold.

3.3 Safety:

The safety parameters (AST, ALT, ALP, and SC levels) were checked for the treatment group at baseline, after the first follow-up at 28 ± 2 days, and after the last follow-up at 84 ± 2 days. All treatment group values remained within normal levels (**Table 5**). No severe adverse events were reported on SAE forms or in interviews. Three individuals in the treatment group self-reported mild nausea or vomiting in the first week of intervention, which was verified at the first follow-up at 28± 2 days. All three cases were self-limiting and did not cause any participant to drop out of the study.

Table 5

Changes in the safety parameters ALP, AST, ALT, and SC at baseline (at Day 0) and after intervention (at Day 84) in the treatment group, mean ± SD

Parameters	Treatment Group (n=17)	Treatment Group (n=17)
	Day 0	Day 84
ALP (U/L)	79.00± 2.18	73.65± 5.35
AST (U/L)	30.00± 1.73	27.82± 2.60
ALT (U/L)	31.41± 1.58	29.41± 3.52
SC (mg/dL)*	17.94± 2.16	17.47± 2.00

**Measured as Blood Urea Nitrogen to Serum Creatinine Ratio.*

4. Discussion

Prediabetic individuals are at higher risk of progressing to type 2 diabetes mellitus and resulting cardiovascular complications such as myocardial infarction and cardiovascular death. They are more prone to nephropathy, neuropathy, and retinopathy as a result of diabetic comorbidities[18], [19].

Dietary supplements are commonly used by a large population around the globe for a variety of health-related goals. In the case of prediabetic individuals, glycemic control and lifestyle modifications remain the mainstay in preventing and delaying its progression to diabetes. With limited proven pharmacological agents, dietary supplementation is increasingly being considered an early intervention for the prevention of prediabetes[20].

Berberine is a natural extract of *Coptis chinensis*, *Berberis aristata,* and Phellodendron bark, has been used for the symptoms of diabetes[21]. Various *in vivo* studies have shown promising improvements in diabetes treatment [22]. Berberine-mediated activation of protein kinase (AMP-activated protein kinase) is partially responsible for the hypoglycemic effect and insulin sensitivity [23], [24].

The present study was conducted to explore the efficacy of HIMABERB® on glycemic control markers in otherwise healthy prediabetic individuals. In the present study, HOMA-IR was also significantly reduced in the treatment group. The effects observed in our study were significantly higher than those in a previous study, which may be attributable to a higher dosage in our study of 500 mg thrice daily versus 300 mg thrice daily [25]. The outcomes are consistent with the results obtained by Wang et al in 2020, which demonstrated a significant reduction in fasting glucose, 2h-OGTT, and HbA_{1c} in prediabetic individuals[25]. A consistent decline in glycemic markers was observed over time, with no deviation of safety parameters outside the normal range. The 500 mg of HIMABERB ® was administered thrice daily for 12 weeks to prediabetes, it was safe and effective in decreasing glycemic control markers (**Figure 2** and **Table 5**).

In a meta-analysis and review of 17 randomized controlled trials on berberine for the treatment of diabetes mellitus, Wei XC et al. [13] concluded that berberine significantly reduces glycemic control markers, including FPG, postprandial blood glucose, and HOMA-IR, in comparison to a control group of placebo or no intervention with medicine [14], [26]–[31]. The results of this double-blind, randomized, placebo-controlled trial demonstrate the effectiveness of HIMABERB® in prediabetes and are in alignment with previous studies on diabetic individuals.

In 2019, Friedman et al. conducted a study to assess the effect of polyherbal supplements on prediabetic adults. The supplement was a combination of cinnamon bark, banana leaf, kudzu root, fenugreek seed, gymnema leaf, and berberine hydrochloride. The researchers concluded that herbal supplements, in the combination above, can be used as an adjunct in preventing progression to type 2 diabetes mellitus[32], [33].

The intervention used in the present study contained only HIMABERB® (a high purity and water-extracted berberine hydrochloride) without any excipients. Strict adherence to the protocol was maintained in this double-blinded, randomized, placebo-controlled trial, with regular follow-ups on participants' supplements and diet diaries. Berberine has been associated with gastrointestinal discomfort and adverse events, including nausea, vomiting, diarrhea, constipation, and abdominal discomfort[17]. Consistent with these findings, three mild cases of nausea or vomiting were seen in this study. No clinically relevant changes were seen in vital signs or levels of AST, ALP, ALT, or SC, which remained within normal limits, indicating that the supplement is safe and well tolerated (**Table 5**).

Treatment with HIMABERB® over the course of 84 days resulted in decreases in mean FPG and HbA1c to below the clinically defined thresholds for prediabetes. These results are clinically meaningful and have the potential to impact the management of patients that are classified as prediabetic. Control of blood glucose in prediabetics to below clinical thresholds using natural and non-toxic agents, as demonstrated here, may benefit disease outcomes and safety for patients.

While statistically and clinically significant effects of HIMABERB® on glycemic markers in prediabetic patients were clear, this study was limited by a small sample size, follow up that was limited to 84 days, single-institution design, and lack of analysis of durability and dose-dependency. A multicentric long-term study with a larger sample size may provide more robust data that is generalizable to the broader population.

5. Conclusion

Intervention with HIMABERB® in prediabetic individuals for 84 days (12 weeks) significantly affected glycemic control markers, supporting the investigation of its use to delay progression to diabetes mellitus. The results suggest that treatment is safe and effective in controlling glycemic control markers in prediabetic patients. Further large multicenter trials are warranted.

Declarations

Ethics approval and informed consent: The work described was carried out by The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans. This study (approval no. SOA/IDS/IRB/2021-10) was approved by the INSTITUTIONAL ETHICAL COMMITTEE, SIKSHA'O'ANUSANDHAN (DEEMED TO BE UNIVERSITY), K-8 KALINGA NAGAR. BHUBANESWAR, ODISHA-75003 on May 17, 2021. The study protocol was registered at http://ctri.nic.in (CTRI/2021/12/038751, December 20, 2021). Informed consent was obtained from all subjects.

Consent for publication: No information regarding specific individuals is included in this article. Informed consent for the study and publication was obtained prior to enrollment.

Availability of data and materials: The datasets used and/or analyzed during the current study and the full trial protocol are available from the corresponding author on reasonable request.

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Figures

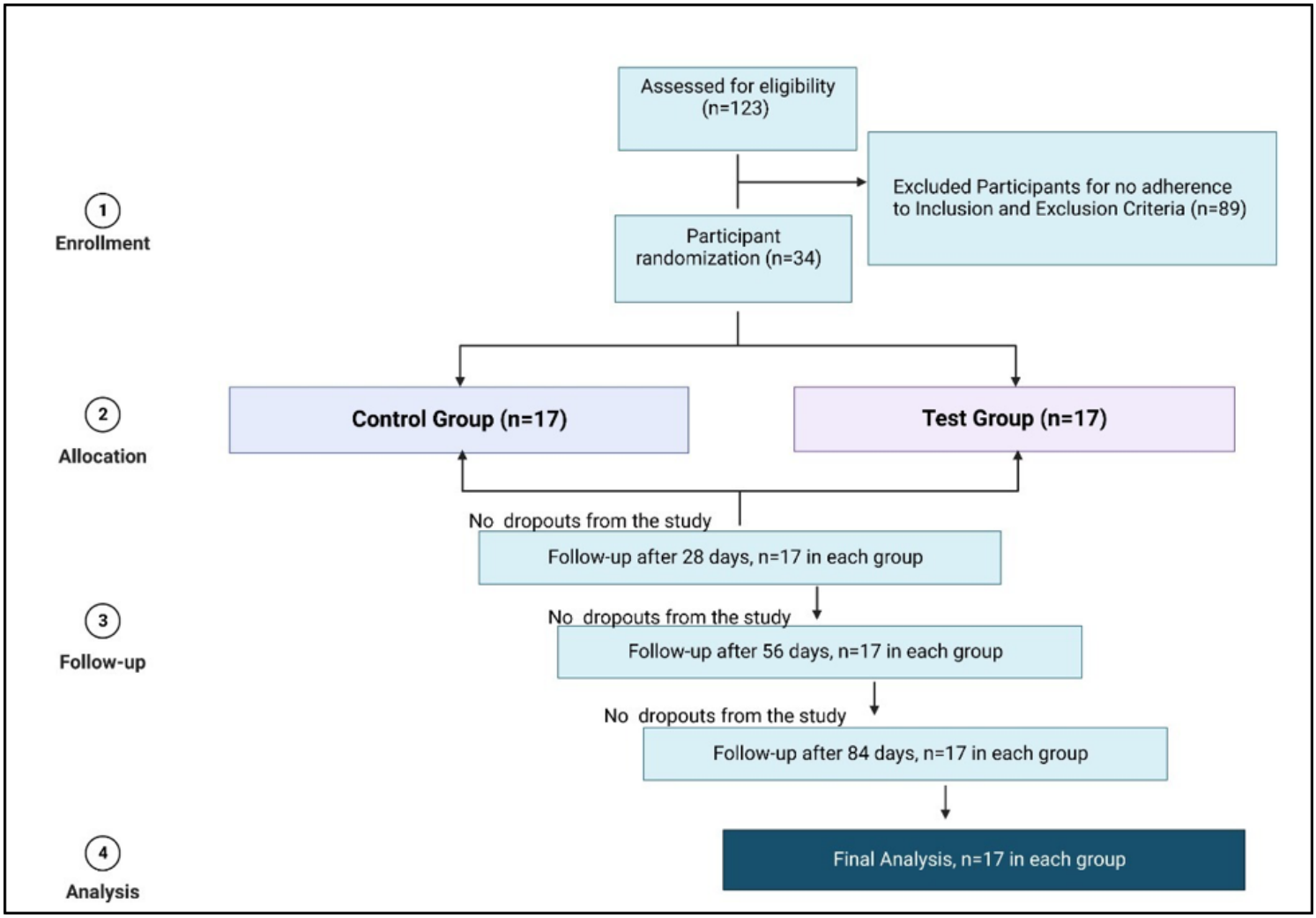

Figure 1

Flow chart of the study design, enrollment, randomization, follow-up, and analysis of study participants

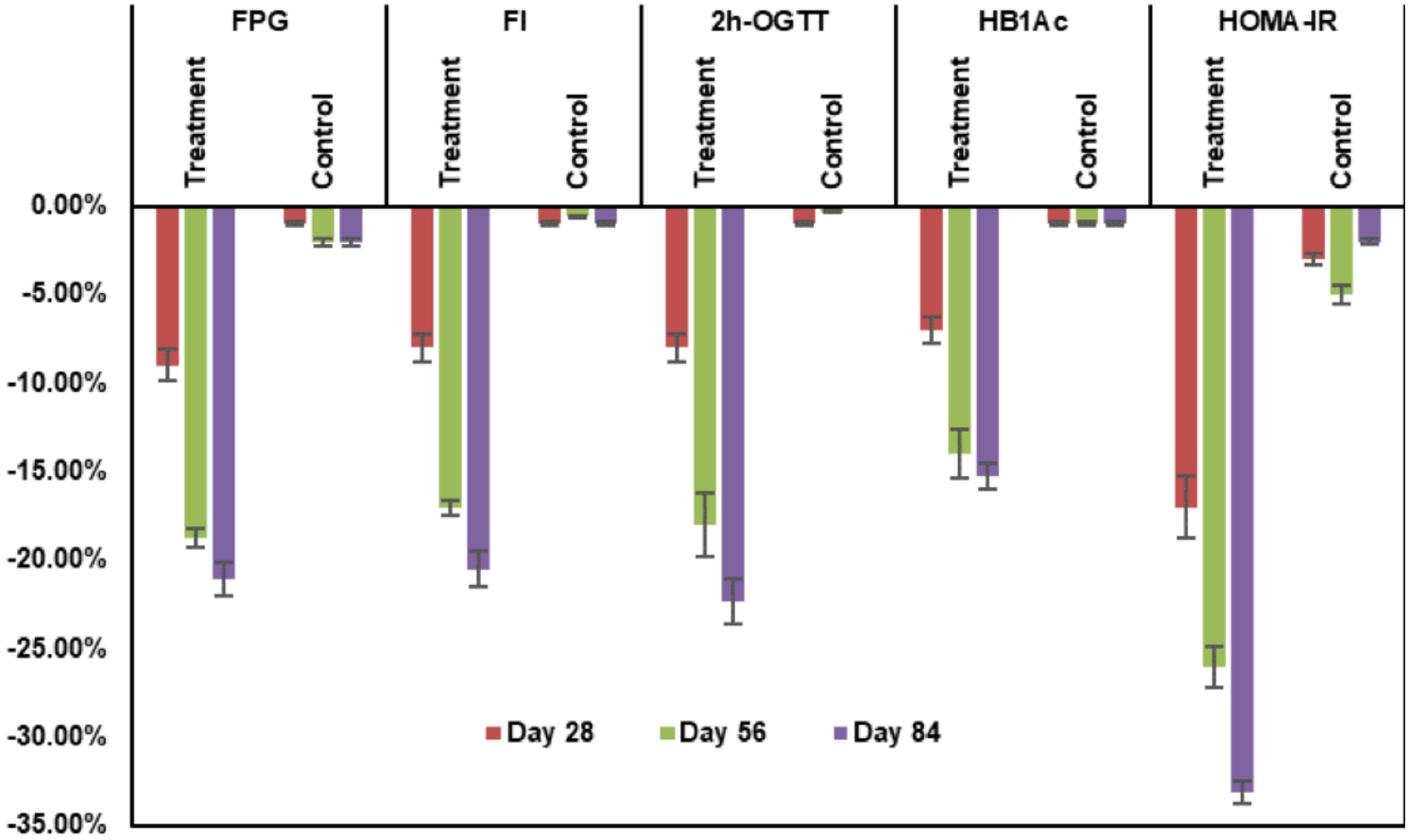

Figure 2

Changes in glycemic control markers (mean ± SD) in the treatment and control groups at different time points across FPG (mmol/L), FI (μIU/mL), 2h-OGTT (mmol/L), HbA_{1c} (%), and HOMA-IR (units). Error bars indicate one standard deviation from the mean.