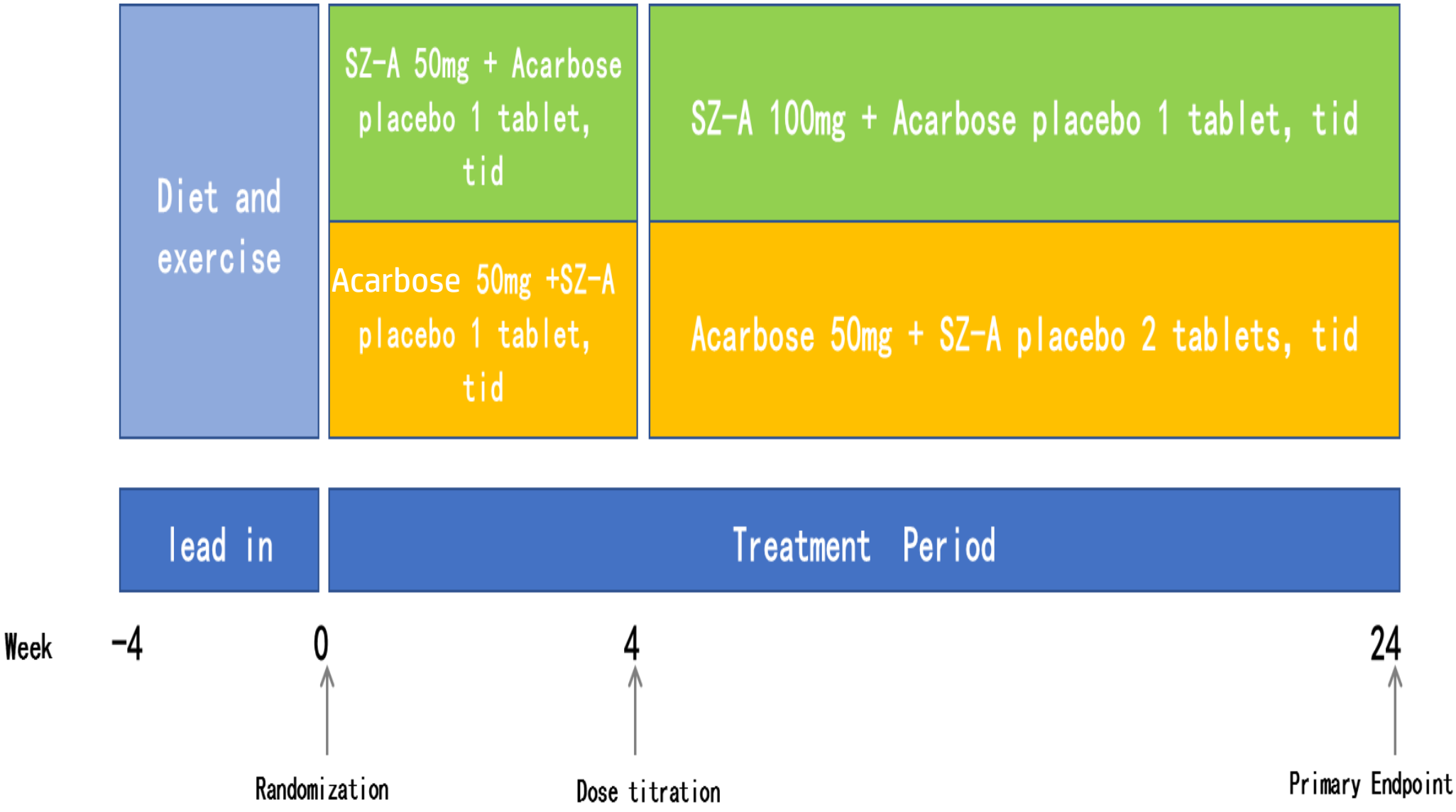
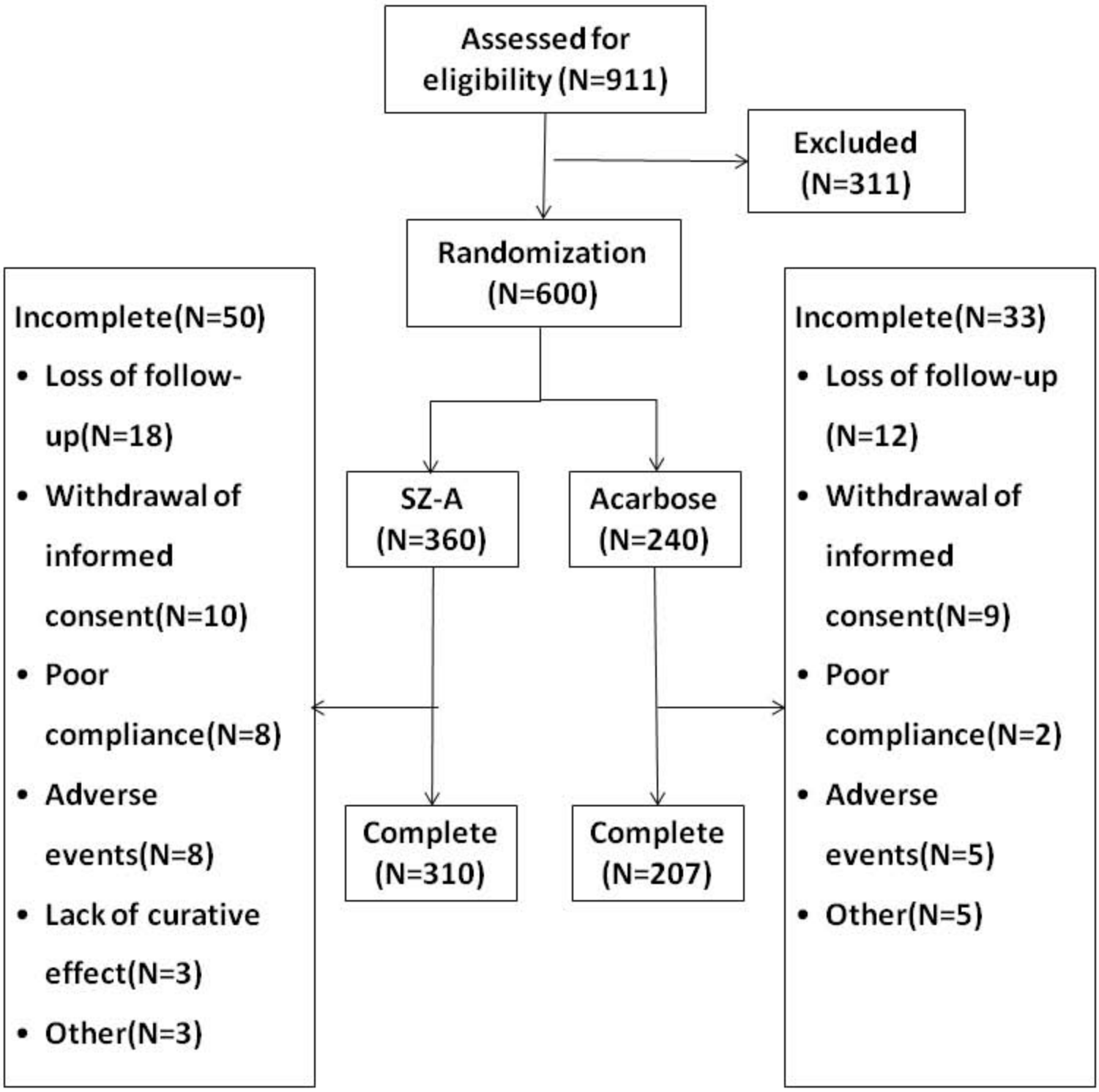
Supplementary Figure 1



Supplementary Fig 2

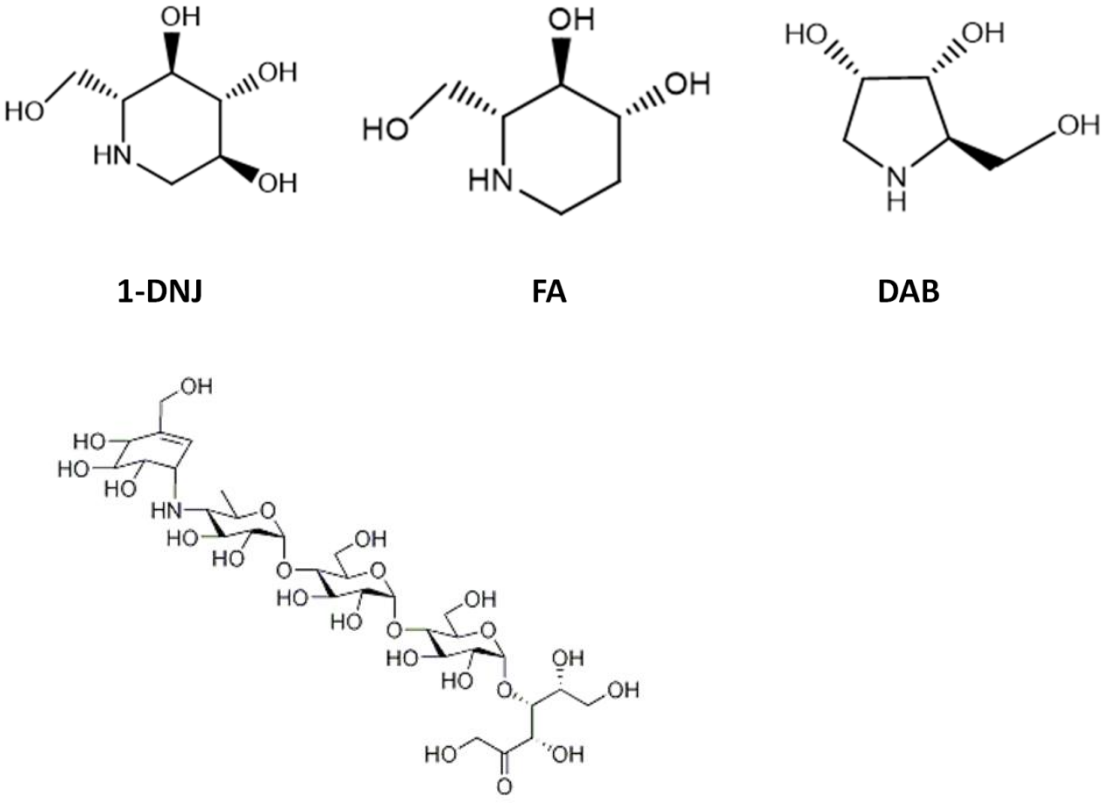


Groups	FAS	PPS	SS
SZ-A	321	261	352
Acarbose	222	185	236

FAS, full analysis set; PPS, per-protocol set; SS, security data set; SZ-A, mulberry twig alkaloids.

1. The main alkaloid ingredients of SZ-A consist of 1-DNJ, FA, and DAB.

SZ-A are the effective alkaloid ingredients of the traditional Chinese medicine *ramulus mori*, which is obtained by extraction and separation, and no chemical synthesis or biological fermentation is involved in the process. *Ramulus mori* comes from the branches of Moraceae plant mulberry, which is widely planted and used in the silkworm industry in China. The leaves of mulberry are used to rear silkworm, while the branches become residual products without absolute usage. Mulberry trees require pruning at least twice per year, resulting in large amounts of cut branches, the medicinal materials to extract and separate SZ-A are sustainable and sufficient. The main alkaloid ingredients of SZ-A include 1-DNJ, FA, and DAB.



Supplementary Figure 3. The chemical structures of 1-DNJ, FA, DAB, and acarbose

1-DNJ: 1-Deoxyno-jirimycin, FA: Fagomine, DAB: 1,4-Dideoxy-1,4-iminod-d-arabinitol

2. Rationale for dosing of Mulberry twig alkaloids

The results of the phase II clinical study were as follows:

1) The low-dose SZ-A group (1 tablet/time) had clinical efficacy and certain clinical value in reducing glycosylated hemoglobin and blood glucose. The medium-dose (2 tablets/time) and high-dose (3 tablets/time) SZ-A groups had the same efficacy as the acarbose group (1 tablet/time).

2) There was a correlation between the incidence of gastrointestinal adverse reactions and dose increase. The adverse reaction rates of the low- and medium-dose SZ-A tablets were lower than those of acarbose tablets, and the adverse reaction rates of the high-dose group were similar to that of acarbose tablets.

3) The gastrointestinal adverse reactions of the SZ-A tablets mainly occurred within four weeks after taking the drug, with tolerance improving over time.

Based on the above three points of the clinical protocol of this study, there was a need to establish a regimen with lower occurrence of gastrointestinal adverse reactions and higher drug compliance to ensure clinical efficacy of the SZ-A tablets. The choice of drug regimen was divided into two stages. In the first stage, one SZ-A tablet tid was given for four weeks, and in the second stage, two tablets tid were given from the fourth week to the end of treatment.

3. Rationale for dosing of acarbose

To select the acarbose dose, an extensive literature search was performed. After considering dose-efficacy relationship, safety profile, the results of high-quality meta-analyses, the real situation of post-marketing usage of acarbose in the Asian population, and relevant information in FDA-approved instructions, acarbose 50 mg tid was used as the control group.

A Cochrane systematic retrospective study of α-glucosidase inhibitors was conducted [1] to investigate the effects of single α-glucosidase inhibitors on glycemic control and their safety in patients with type 2 diabetes mellitus. A total of 41 clinical research reports were collected, covering patients in Asia, Europe, America, and other countries, of which 30 involved acarbose. Acarbose at the different doses, 50, 100, 200, and 300 mg tid, can reduce glycosylated hemoglobin by 0.90%, 0.76%, 0.77%, and 0.78%, respectively, suggesting that even when the dose is increased beyond 50 mg tid, there was no significant difference in clinical efficacy, but adverse reactions odds ratio(OR) significantly increased. The results are shown in the table below:

Changes in HbA1c and adverse reactions OR in different doses of acarbose compared with placebo				
Index	**50 mg tid**	**100 mg tid**	**200 mg tid**	**300 mg tid**
HbA1c	−0.9%	−0.76%	−0.77%	−0.78%
OR	1.95	4.12	6.97	8.31

A randomized double-blind study on the efficacy of different doses of acarbose in Europe [2], which included 475 patients with type 2 diabetes who had poor diet control, 81 patients with placebo, 86 patients with acarbose at 25 mg tid, 88 patients with acarbose at 50 mg tid, and 88 patients with acarbose at 100 mg tid. The results were as follows:

Variable	**Placebo**	**25 mg**	**50 mg**	**100 mg**
HbA1c	0.37%	−0.05%	−0.44%	−0.45%
Difference from placebo		−0.42%	−0.81%	0.82%

Another multicenter randomized double-blind placebo-controlled clinical study completed in the United States [3] compared the safety and effectiveness of different doses of acarbose in the treatment of type 2 diabetes in a course of 22 weeks. There

were 64 cases of placebo, 58 cases of acarbose 100 mg/tid, and 54 cases of 200 mg/tid. The results were as follows:

Variable	Placebo	100 mg	200 mg
HbA1c	0.33%	−0.45%	−0.4%
FBG mmol/L	1.1	−0.41	−1.06
PPG 1h mmol/L	1.77	−2.36	−2.92
PPG 2h mmol/L	1.65	−2.12	−3.17

The results of the above randomized double-blind dose-efficacy relationship study and meta-analysis indicated that the efficacy at a dose of 50 mg tid met the clinical requirements. The therapeutic effect of acarbose at a dose higher than 50 mg tid reached a plateau, and the therapeutic effect did not increase with higher doses.

Among the post-marketing studies in Asia and China, one was a multi-center large-scale post-marketing clinical observation in the Asia-Pacific region [5] that studied the efficacy and tolerance of acarbose as an additive or monotherapy for patients with type 2 diabetes. A total of 14,574 patients were included, and the average daily dose of acarbose was 115.6 mg at the beginning and was increased to 139.4 ± 65.1 mg at the third follow-up. A total of 10,009 patients (69.4%) maintained the same dose during the study period as at the initial visit. The other was the post-marketing re-evaluation study of acarbose for the treatment of T2DM in China, with the participation of 133 Chinese doctors and enrolled a total of 2,550 cases (88.2% were type 2 diabetes, 11.8% were IGT); 77% of the diabetic patients were consistently given a maintenance dose of 50 mg tid. The application characteristics at this dose were concordant with the results of large-scale post-marketing studies in the United States and Germany [7,8].

In addition, changes in HbA1C at different doses of acarbose are referenced in the FDA instructions:

Dose	Case load	Change in HbA1c (%)	P value (compared with placebo)
25 mg tid	110	−0.44	0.0307
50 mg tid	131	−0.77	0.0001
100 mg tid	244	−0.74	0.0001

The results of HbA1c changes showed that a low dose of 25 mg tid imparts a definite therapeutic effect. When the dose was increased to 50 mg tid, the therapeutic effect significantly improved, but when it was further increased to 100

mg tid, the therapeutic effect reached a plateau, and there was no significant difference in the changes in HbA1c. However, when the dosage reached 100 mg tid, the incidence of adverse reactions increased.

Therefore, based on the above reasons, the treatment dose of acarbose was set at 50 mg tid in this study.

References

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[4] Zhang W, Kim D, Philip E, et al. A multinational, observational study to investigate the efficacy, safety and tolerability of acarbose as add-on or monotherapy in a range of patients: the Gluco VIP study. *Clinical Drug Investigation* Apr 2013;33(4):263-274.

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[7] Mertes G. Safety and efficacy of acarbose in the treatment of type 2 diabetes data from a 5-year surveillance study. *Diabetes Research and Clinical Practice* 2001;52:193–204.

[8] The FDA instructions of acarbose.

4. Detection of HbA1c

The central laboratory is responsible for the detection of HbA1c from day 0 of baseline to 24 weeks after treatment. Each center operated in accordance with the standard operating procedures for the collection and transportation of HbAlc blood samples in advance. To detect HbA1c levels, the instrument, American BIO-RAD Variant II, at the central laboratory was used for high-performance liquid chromatography. The biological samples for testing were stored using special blood collection tube (Hemoglobin Capillary Collection System) for the BIO-RAD Variant II. The blood samples in the blood collection tube were collected by each center in accordance with the provisions of the plan, and then transferred by specified cold-chain transportation or inspectors in the same city to the central laboratory. After the test, the remaining samples and backup samples were returned to the sponsor for preservation for future reference. The central laboratory was authenticated for testing and provided the standard for quality control.

5. Assessment of postprandial 1h-PBG and 2h-PBG

Assessment of postprandial 1h-PBG and 2h-PBG was performed by glucose tolerance test after the unified standard diet. The details of the method are as follows: subjects were required to eat a unified standard meal provided by the researcher within 10 min, then venous blood samples were collected at the specified time point to assess venous plasma glucose levels.

6. Judgment criteria for adverse events

In this study, severe adverse events (SAEs) refer to events that occur during the clinical trial that require hospitalization (the patients with type 2 diabetes who are hospitalized due to poor glycemic control were also defined as SAEs), prolong hospitalization, result in disability, affect work ability, are life-threatening or result in death, or cause congenital malformations.

A meeting for data review was held by the main investigators, sponsors, statistical analysts, and data management personnel, and the data were blind reviewed and assessed in terms of a causal relationship with the drug. Determination principle: According to the causality assessment system, the likelihood of the relationship with a drug can be classified into five categories: 1 - Certain; 2 - Probably/likely; 3 - Possible; 4 - Unlikely; 5 - Uncertain. The assessment criteria of causal categories were as follows: (1) Whether there is a reasonable relationship between initiation of treatment and the occurrence of suspected adverse reactions; (2) Whether the suspected adverse reactions meet the known adverse reactions of the drug; (3) Whether the suspected adverse reactions can be explained by the combined therapy, the clinical conditions of patients, or the influence of other therapies; (4) Whether the suspected adverse reaction disappeared or was alleviated after drug withdrawal or dosage reduction; (5) Whether the same reaction reappeared after a subsequent exposure to the suspected drug.

Result	Judgement criterion				
	①	②	③	④	⑤
Certain	+	+	-	+	+
Probably/likely	+	+	-	+	?
Possible	+	+	+	±	?
Unlikely	+	-	±	±	?
Uncertain	-	-	+	-	-

Note: “+”, affirmative, “-” represents negative, “±” indicates that it is difficult to affirm or deny, and “?” represents unknown.

The relationship between adverse events (including SAEs) and drugs should be determined as much as possible. If it is judged to be certain, probably/likely, and possible, then it will be regarded as a treatment-related adverse events (TAE) and evaluated as an SAE according to severity.

According to the above criteria, SAEs were observed in the SZ-A and acarbose groups in this study. However, these SAEs were determined by investigators and independent experts without unblinding that they were not related to drug treatment, and there was no significant difference in incidence between the two groups.

7. ITT analysis

ITT analysis showed that the mean change in HbA1c levels after 24 weeks of treatment in the SZ-A and acarbose groups was −0.92% (−10.1 mmol/mol, N=360) and −0.89% (−9.7 mmol/mol, N=240), respectively. The mean difference between the SZ-A and acarbose groups (least squares mean difference) was −0.03% (95% CI: −0.15% to 0.08%) (0.3 mmol/mol, [95% CI: −1.6 to 0.9]). According to the non-inferior standard of 0.3% (3.3 mmol/mol), the SZ-A group was not inferior to the acarbose group. The results of ITT analysis, full analysis set, and per-protocol set analysis were similar.

8. Adverse events (SS)

System organ classification (SOC) Preferred term (PT)	SZ-A group (N=352) Number (%)	Acarbose group (N=236) Number (%)
Total	**189 (53.7)**	**135 (57.2)**
Gastrointestinal diseases	**56 (15.9)**	**62 (26.3)**
Flatulence	20 (5.7)	31 (13.1)
Abdominal distension	12 (3.4)	16 (6.8)
Diarrhea	11 (3.1)	12 (5.1)
Infection and infectious diseases	**61 (17.3)**	**45 (19.1)**
Urinary tract infection	30 (8.5)	20 (8.5)
Upper respiratory tract infection	24 (6.8)	16 (6.8)
Metabolic and nutritional diseases	**50 (14.2)**	**27 (11.4)**
Hyperuricemia	32 (9.1)	14 (5.9)
Laboratory examinations	**44 (12.5)**	**28 (11.9)**
Elevated alanine aminotransferase	3 (0.9)	0 (0)
Increased blood creatinine	3 (0.9)	1 (0.4)
Hepatobiliary system disease	**13 (3.7)**	**6 (2.5)**
Abnormal liver function	9 (2.6)	4 (1.7)
Liver steatosis	0 (0)	2 (0.8)
Nonalcoholic steatohepatitis	1 (0.3)	0 (0)
liver damage	1 (0.3)	0 (0)
Acute cholecystitis	1 (0.3)	0 (0)
Drug-induced liver injury	1 (0.3)	0 (0)
Heart disease	**8 (2.3)**	**6 (2.5)**
Kidney and urinary system diseases	**6 (1.7)**	**0 (0)**

SS: security data set

9-1. Severe adverse events (Summary, SS)

System organ classification (SOC) Preferred term (PT)	SZ-A group (N=352) Number (%)	Acarbose group (N=236) Number (%)
Total	**19 (5.4)**	**9 (3.8)**
Nervous system disorders	2 (0.6)	3 (1.3)
Cerebral infarction	0 (0)	1 (0.4)
Cerebral embolic stroke	1 (0.3)	0 (0)
Embolic stroke	1 (0.3)	0 (0)
Diabetic neuropathy	0 (0)	1 (0.4)
Dizziness	0 (0)	1 (0.4)
Musculoskeletal and connective tissue disorders	3 (0.9)	1 (0.4)
Slipped disk	2 (0.6)	0 (0)
Myasthenia	0 (0)	1 (0.4)
Wrist fracture	1 (0.3)	0 (0)
Lumbar spinal stenosis	1 (0.3)	0 (0)
Metabolism and nutrition disorders	1 (0.3)	2 (0.8)
Patients with type 2 diabetes who hospitalized due to poor glycemic control	0 (0)	2 (0.8)
Diabetic ketoacidosis	1 (0.3)	0 (0)
Hepatobiliary disorders	3 (0.9)	0 (0)
Nonalcoholic steatosis hepatitis (NASH)	1 (0.3)	0 (0)
Liver dysfunction	1 (0.3)	0 (0)
Acute cholecystitis	1 (0.3)	0 (0)
Respiratory, thoracic, and mediastinal disorders	2 (0.6)	1 (0.4)
Pneumonia	1 (0.3)	0 (0)
Cough	1 (0.3)	0 (0)

System organ classification (SOC) Preferred term (PT)	SZ-A group (N=352) Number (%)	Acarbose group (N=236) Number (%)
Cheat discomfort	0 (0)	1 (0.4)
Infections and infestations	2 (0.6)	0 (0)
Amygdalitis	1 (0.3)	0 (0)
Tracheobronchitis	1 (0.3)	0 (0)
Investigations	2 (0.6)	0 (0)
Blood in the urine	1 (0.3)	0 (0)
Abnormal eosinophil count	1 (0.3)	0 (0)
Neoplasms benign, malignant and unspecified (including cysts and polyps)	0 (0)	2 (0.8)
Lung squamous cell carcinoma	0 (0)	1 (0.4)
Liver metastases	0 (0)	1 (0.4)
Papillary thyroid carcinoma	0 (0)	1 (0.4)
Cardiac disorders	2 (0.6)	0 (0)
Ventricular arrhythmias	1 (0.3)	0 (0)
Angina pectoris	1 (0.3)	1 (0.4)
Immune system disorders	0 (0)	1 (0.4)
Autoimmune thyroiditis	0 (0)	1 (0.4)
Reproductive system and breast disorders	1 (0.3)	0 (0)
Benign prostatic hyperplasia	1 (0.3)	0 (0)
Gastrointestinal disorders	1 (0.3)	0 (0)
Enteritis	1 (0.3)	0 (0)
Vascular disorders	1 (0.3)	0 (0)
Arteriostenosis	1 (0.3)	0 (0)
Eye disorders	0 (0)	1 (0.4)
Keratitis	0 (0)	1 (0.4)

SS: Security data set

9-2. Severe adverse events (Monotherapy, SS)

System organ classification (SOC) Preferred term (PT)	SZ-A group (N=177) Number (%)	Acarbose group (N=117) Number (%)
Total	**12 (6.8)**	**4 (3.4)**
Nervous system disorders	1 (0.6)	1 (0.9)
Embolic stroke	1 (0.6)	0 (0)
Dizziness	0 (0)	1 (0.9)
Musculoskeletal and connective tissue disorders	2 (1.1)	1 (0.9)
Myasthenia	0 (0)	1 (0.9)
Wrist fracture	1 (0.6)	0 (0)
Lumbar spinal stenosis	1 (0.6)	0 (0)
Metabolism and nutrition disorders	1 (0.6)	1 (0.9)
Patients with type 2 diabetes who hospitalized due to poor glycemic control	0 (0)	1(0.9)
Diabetic ketoacidosis	1 (0.6)	0 (0)
Hepatobiliary disorders	1 (0.6)	0 (0)
Nonalcoholic steatosis hepatitis (NASH)	1 (0.6)	0 (0)
Respiratory, thoracic, and mediastinal disorders	2 (1.1)	0 (0)
Pneumonia	1 (0.6)	0 (0)
Cough	1 (0.6)	0 (0)
Infections and infestations	2 (1.1)	0 (0)
Amygdalitis	1 (0.6)	0 (0)
Tracheobronchitis	1 (0.6)	0 (0)
Investigations	2 (1.1)	0 (0)
Blood in the urine	1 (0.6)	0 (0)
Abnormal eosinophil count	1 (0.6)	0 (0)

System organ classification (SOC) Preferred term (PT)	SZ-A group (N=177) Number (%)	Acarbose group (N=117) Number (%)
Neoplasms benign, malignant and unspecified (including cysts and polyps)	0 (0)	2 (1.7)
Lung squamous cell carcinoma	0 (0)	1 (0.9)
Liver metastases	0 (0)	1 (0.9)
Papillary thyroid carcinoma	0 (0)	1 (0.9)
Immune system disorders	0 (0)	1 (0.9)
Autoimmune thyroiditis	0 (0)	1 (0.9)
Gastrointestinal disorders	1 (0.6)	0 (0)
Enteritis	1 (0.6)	0 (0)
Vascular disorders	1 (0.6)	0 (0)
Arteriostenosis	1 (0.6)	0 (0)

SS: Security data set

9-3. Severe adverse events (Combined with metformin, SS)

System organ classification (SOC) Preferred term (PT)	SZ-A group (N=175) Number (%)	Acarbose group (N=119) Number (%)
Total	**7 (4.0)**	**5 (4.2)**
Nervous system disorders	1 (0.6)	2 (1.7)
Cerebral infarction	0 (0)	1 (0.8)
Cerebral embolic stroke	1 (0.6)	0 (0)
Diabetic neuropathy	0 (0)	1 (0.8)
Musculoskeletal and connective tissue disorders	1 (0.6)	0 (0)
Slipped disk	1 (0.6)	0 (0)
Lumbar spinal stenosis	1 (0.6)	0 (0)
Metabolism and nutrition disorders	0 (0.3)	1 (0.8)
Patients with type 2 diabetes who hospitalized due to poor glycemic control	0 (0)	1 (0.8)
Hepatobiliary disorders	2 (1.1)	0 (0)
Liver dysfunction	1 (0.6)	0 (0)
Acute cholecystitis	1 (0.6)	0 (0)
Respiratory, thoracic, and mediastinal disorders	0 (0)	1 (0.8)
Cheat discomfort	0 (0)	1 (0.8)
Cardiac disorders	2 (1.1)	0 (0)
Ventricular arrhythmias	1 (0.6)	0 (0)
Angina pectoris	1 (0.6)	0 (0)
Reproductive system and breast disorders	1 (0.6)	0 (0)
Benign prostatic hyperplasia	1 (0.6)	0 (0)
Eye disorders	0 (0)	1 (0.8)
Keratitis	0 (0)	1 (0.8)

SS: Security data set

10. Treatment-related adverse events (SS)

System organ classification (SOC) Preferred term(PT)	SZ-A group (N=352) Number (%)	Acarbose group (N=236) Number (%)
Total	**54 (15.3)**	**67 (28.4)**
Gastrointestinal diseases	**43 (12.2)**	**58 (24.6)**
Flatulence	20 (5.7)	31 (13.1)
Abdominal distension	12 (3.4)	16 (6.8)
Diarrhea	9 (2.6)	11 (4.7)
Metabolic and nutritional diseases	**12 (3.4)**	**6 (2.5)**
Hyperuricemia	5 (1.4)	1 (0.4)
Laboratory examinations	**4 (1.1)**	**5 (2.1)**
Increased blood creatinine	2 (0.6)	1 (0.4)
Elevated alanine aminotransferase	1 (0.3)	0 (0)
Hepatobiliary system disease	**0 (0)**	**3 (1.3)**
Abnormal liver function	0 (0)	3 (1.3)
Various neurological diseases	**2 (0.6)**	**0 (0)**
Dizziness	2 (0.6)	0 (0)
Systemic diseases and various reactions at the site of administration	**0 (0)**	**2 (0.8)**
Infection and Infectious diseases	**1 (0.3)**	**0 (0)**
Kidney and urinary system diseases	**0 (0)**	**0 (0)**

SS: Security data set

11. Abbreviations tables

1-DNJ	**1-Deoxyrijolymycin**
1h-PBG	**1 Hour-postprandial blood glucose**
2h-PBG	**2 Hour-postprandial blood glucose**
AEs	**Adverse events**
$AUC_{0\text{-}2h}$	**Area under the curve of PBG**
BMI	**Body mass index**
DAB	**1,4-Dideoxy-1,4-imino-D-arabitol**
DAS	**Data analysis system**
FA	**Fagomine**
FAS	**Full analysis set**
FBG	**Fasting blood glucose**
GDs	**Gastrointestinal disorders**
HbA_{1c}	**Glycosylated hemoglobin**
ITT	**Intention-to-treat**
PPS	**Per-protocol set**
RCT	**Randomized controlled trial**
SAEs	**Severe adverse events**
SD	**Standard deviation**
SS	**Security data set**
SZ-A	**Mulberry twig alkaloids**
T2D	**Type 2 diabetes**
TAEs	**Treatment-related adverse events**