Comparison the effects of aqueous and ethanolic extracts of Rheum ribes on insulin resistance and apolipoprotein AI (ApoA1), apolipoprotein B (ApoB) and Apo B to ApoA1 ratio in patients with type 2 diabetes mellitus: A Randomized, Double-Blind, and Placebo-Controlled Clinical Trial

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Abstract

Background: This study was conducted to determine the effect of Rheum ribes supplementation on glycemic indices and apolipoproteins in patients with type 2 diabetes mellitus (DMT2).

Methods: In this randomized controlled trial, sixty type 2 diabetic patients, aged 30-60 years with body mass index (BMI) of 20-30 kg/m 2 , and hemoglobin A1c (HbA1c) of 6-8% were included. The patients were randomly assigned to receive 450 mg of Rheum ribes aqueous extract (AG), 450 mg of Rheum ribes ethanolic extract (EG) or placebo (PG), three times daily for 6 weeks. Then glucose, the homeostatic model assessment (HOMA-IR and HOMA-B) and apolipoprotein A-I (ApoA1) and apolipoprotein B (ApoB) were measured.

Results: According to these findings, in the AG and EG intervention groups, we observed a significant reduction in serum levels of insulin (P=0.003 and P=0.001, respectively), HOMA-IR (P=0.01 and P=0.001, respectively) and HOMA-B (P=0.002 and P=0.001, respectively) indices, without no significant changes in glucose. There was also a significant reduction in serum levels of ApoB (P=0.006 and P=0.03, respectively) and ApoB/ApoA1 ratio (P=0.016 and P=0.04, respectively) in both AG and EG. Intervention in both AG and EG had increasing effects on ApoA1 (P=0.08 and P=0.05, respectively). None of these variables had a significant change in PG. At the end of study, there were significant differences in insulin (P=0.04), HOMA-IR (P=0.03), HOMA-B (P=0.01), ApoB (P=0.02), and ApoB/ApoA1 (P=0.03) ratio among groups.

Conclusions: Rheum ribes intake may have favorable effects on insulin resistance and apolipoproteins in diabetic patients.

Trial registration: The study was recorded in Iranian Registry of Clinical Trials under the registration number of IRCT201410142709N31 (Registration date: 2014-12-11, https://en.irct.ir/trial/2543).

Background

Diabetes mellitus is a long-term metabolic disorder that is characterized by hyperglycemia due to impairment of insulin secretion, insulin function or both. Type II diabetes mellitus (DMT2) is a kind of diabetes which affects 90–95% of diabetic patients and, the treatment of which is insulin-independent. The signs and symptoms of this disease are the elevated serum levels of glucose, decreased peripheral absorption of glucose due to impairment of insulin secretion and peripheral resistance to insulin (1).

During the last decade, the prevalence of DMT2 has increased. DMT2 is a major risk factor for developing cardiovascular diseases such as coronary vascular diseases or congestive heart failure, chronic neural, renal and optic diseases and also non-healing wounds that occur due to hyperglycemia. This disease imposes a huge expense on society such that all countries throughout the world spend the 5–13% of the costs allocated to disease treatment for diabetes (2, 3).

The first step in the management of diabetes is to control the serum levels of glucose via many ways including the use of hypoglycemic drugs, physical activity and insulin therapy (4). In one study, it was seen that patients need to use insulin therapy after 10 years of use of hypoglycemic drugs. Insulin therapy has some side effects, including lipohypertrophy and lipoatrophy (5).

Herbal medicine is one way of managing diabetes (6). Until now, many plants have been tested and used in the prevention and treatment of diabetes. According to the literature, more than 800 plants are discovered to have anti-diabetic properties (7).

Some herbs have been tested and approved to protect the reform of ß-cells of the pancreas and improve insulin resistance. Also, some others have anti-hyperlipidemic and antioxidant activity properties which can be very useful in the

treatment of chronic disease. Most of the medicinal plants contain alkaloids, flavonoids, carotenoids, glycosides, terpenoids, etc., that are frequently showed have anti-diabetic effects (8).

Rheum ribes L. belongs to the family of plants that named Polygonaceae (9). It has used for medicinal purposes; such as diabetes, antidiarrheal, gastrointestinal hemorrhage, laxative, and treatment of injuries, also its fresh forms are consumed as a vegetable (10). It is commonly found in Anatolia, Turkey, Iraq, and Iran (11). Some studies reported that the Rheum ribes improves renal failure, modifies the glucose uptake and diabetic nephropathy in experimental designs (12, 13). The extract of Rheum ribes roots possesses significant glucose-lowering activity in induced polycystic ovary syndrome rabbits (14). The Hypocholesterolemic effects of both ethanolic and aqueous extracts of R. ribes in rabbit have been also reported (15).

The extract of Rheum ribes has consisted of many components such as tannins and anthracene derivatives (16). The results of the decomposition of Rheum ribes showed that the derived materials from the plant include substances like physcion, chrysophanol, aloe-emodin rhein, physcion-8-O-glucoside, sennoside A and rhaponticin (13). According to the phenolic constituent profile of Rheum ribes, such as their flavonoids, stilbenes, and anthraquinones, they are a great source of antioxidants (10).

Studies have shown that the serum levels of glycemic indices and lipid profile are changed in patients with DMT2 (17). Although some experimental studies have shown the effects of Rheum ribes on glycemic indices and lipid profile in animal models of DMT2, the Rheum ribes effects on glycemic indices and apolipoprotein A-I (ApoA1), apolipoprotein B (ApoB) and the ApoB/ApoA1 ratio of diabetic patients have not been examined. Hence, the current study aimed to investigate whether the aqueous and ethanolic extract of Rheum ribe improves the glycemic status and apolipoproteins in patients with DMT2. To our knowledge, this is the first study that evaluates two kinds of aqueous and ethanolic Rheum ribes extracts.

Method And Materials

Participants

In a randomized, placebo-controlled, double-blind clinical trial, DMT2 patients aged 30-60 years old were recruited for the current study from Firoozgar hospital, Tehran, Iran. This study was designed with 90% power, with 2- sided a=0.05 (type I error), to detect a 5% difference in serum glucose between the 2 group. On the basis of SDs observed in the current study, the number of subjects needed to treat to detect this difference was 16/group. Given an anticipated dropout rate of 25 percent, we set the enrollment target at 20 subjects. The inclusion criteria were as follows, fasting blood glucose≥126 mg/dl, age of participants rages 30-60 years old and unique medication plan as Metformin+Glibenclamide. The diagnosis was performed by an endocrinologist via fasting blood glucose more than 126 mg/dl. Exclusion criteria were as follows, insulin treatment, sensitivity to the extract of *Rheum ribes*, pregnancy during the study, variation in the dosage of the medicines during the study, consumption of any dietary supplements, smoking, alcoholism, green tea consumption, and use of other herbal medicine. A history of diseases including liver, kidney and cardiovascular diseases, thyroid disorders, gastrointestinal problems, lipid-lowering or anti-hypertension treatment, using corticosteroids, cyclosporine, non-steroidal anti-inflammatory or immunosuppressive drugs, warfarin and anti-epileptic medications, pregnancy or breastfeeding, allergy to plants of ragweed species was taken for each subject.

All participants provided written informed consent after receiving an explanation of the purposes of the study, inclusion criteria, exclusion criteria, disadvantages and advantages of the study which were approved by the ethics committee of the Iran University of Medical Sciences. Meanwhile, all of participants were requested to don't alter their usual dietary intake and physical activity during the study. The study was recorded in the clinical trial record center of Iran under the registration number of IRCT201410142709N31

Plant material and extract preparation

Rheum ribes were obtained from the Shahroud region (Semnan, Iran). The plants were washed, air-dried for 12 hours, and turned into small pieces. Identification of the plant was confirmed at the Department of Medicinal Plants, Faculty of Pharmaceutical Sciences, Shahid Beheshti University of Medical Sciences, Tehran, Iran.

Then, the pieces were equally assigned to each of the ethanolic or aqueous groups and placebo. The extraction was carried out using a maceration method according to previous studies (18). For this purpose, 100 g of dried rhubarb flowers were poured into a dish after grinding, and 1000 ml of ethyl alcohol and 1000 ml of water were added to it. After 4 hours, the contents of the container were stacked with flat paper in a clean lab and then the container was placed on a bain-marie. After evaporation of the solvent, an extract was obtained, as for every 100 grams of rhubarb, 8 grams of extract was obtained. The alcoholic group was immersed in ethanol alcohol in dark and closed containers, and, were stored for 4 days. After that, it was filtered off, the pulp was removed, and the purified extract was evaporated by using a rotary evaporator. Moreover, to prepare an aqueous extract, the immersed plant in water was boiled in the metal tank. Then, it was cool down at the laboratory temperature, passed through filter paper, concentrated on bain-marie. After these steps, extracts were turned in to dry extract, converted into granular and powder by corn starch. Ultimately, the capsulated step was done.

Regarding chemical composition, Rhubarb plant was analyzed in Faculty of Pharmacy, Shahid Beheshti University of Medical Sciences, Tehran, Iran, by High Performance Liquid Chromatography or HPLC analysis method and its constituents were obtained: α-Pinene (13.5%), α-Terpinene (1.3%), p-Cymene (10.6%), Limonene (8.6%), trans-β-Ocimene (1.4%), Terpinolene (12.4%), Isoterpinolene (1.4%), cis-Isopulegone (2.1%), Sabinyl acetate (4.3%), 4-Vinyl-2-methoxy-phenol (2.1%), β-Elemene (1.5%), Germacrene-d (22.3%), Bicyclogermacrene (9.6%), and γ-Dodecadienolactone (3.7%). The others were in trace amounts

Study design

After selecting the samples, a general information questionnaire was completed. Body weight was measured using a scale (Seca, Hamburg, Germany) for all subjects, without shoes and wearing light clothing. Height was measured with a mounted tape and without shoes. BMI was assessed as weight in kilograms divided by height in meters squared.

Information about daily energy and macronutrients intake was obtained by 3-day dietary recall, including 2 regular days and 1 weekend day. Three days average dietary data were analyzed by Nutritionist 4 software for all subjects at the beginning of the study and the end of 6^{th} weeks of study (First Databank Inc., HearstCorp., San Bruno, CA).

At the study baseline and after stratification for gender, pre-intervention weight and age, subjects were randomly assigned to three groups of 20 subjects each as follows: The aqueous extract group (AG, n= 20) and the ethanolic extract group (EG, n= 20) were given 3 *Rheum ribes* capsules daily for 6 weeks. Each capsule contained 450 mg *of Rheum* extract (3×450 mg daily). The placebo group (PG, n= 20) was given 3 placebo capsules (contain starch) daily for the same period which was similar to capsules of AG and EG in appearance, shape, and color. The capsules of *Rheum* ribes was prepared by Department of Medicinal Plant, Faculty of Pharmacy, Shahid Beheshti University of Medical Sciences, Tehran, Iran.

Compliance of the volunteers with the study protocol was evaluated via phone interviews once per week and by counting returned capsules every 2 weeks.

Biochemical assessment

Fasting blood samples (10ml) were drawn from the brachial artery of all participants after 12 hours fasting for biochemistry tests at the beginning and 6^{th} weeks of intervention. Serum samples were immediately centrifuged (Sigma,

UK) at 10000 rpm for 20 min to separate serum. Then the serum samples were stored at -70^{c} before analysis at the Iran University of Medical Sciences reference laboratory. Serum glucose was measured by standard enzymatic method of glucose oxidase. Serum levels of ApoB and ApoA1 were measured by the method of immunoturbidimetry. The serum levels of insulin were measured by the ELISA method. Commercial kits were used to measure serum levels of insulin, glucose, ApoA1 and ApoB (Pars Azmoon, Tehran, Iran). Insulin resistance was assessed by Homeostasis Model Assessment (HOMA-IR) and beta cells function assessed by Homeostasis Model Assessment-beta (HOMA-B) with the formula: [glucose (nmol/L) x insulin (microU/L) /22.5] and [20 x Insulin (μIU/ml) /glucose (mmol/ml) -3.5]%, respectively. Interventions were followed by phone contact.

Statistical methods

Data were analyzed by SPSS software, version 22. In this study, less than 0.05 was considered as a meaningful level. All quantitative variables were reported as mean± SD and qualitative variables were reported as a number (percent). The Kolmogorov–Smirnov test was used for determining the normality of the data. The paired T-test test was used for comparing the mean of pre-intervention and post-intervention data with a normal distribution. The ANOVA method was used for comparing the mean of the three groups. Besides, ANCOVA were used for adjustment of the confounding factors such as age and pre-intervention variants. The method of analysis of variance with repetitive measurements and follow-up tests were used for evaluating the meaningfulness of diet determined by 2 measurements during the intervention period. The confidence level for all variables was considered 95%. The statistically meaningful level was $P<0.05$.

Results

Among 60 participants in the study, all subjects were present until the end of the study, and the side effects of the ethanolic and aqueous extract of rhubarb were not reported. The mean age of subjects under study was 54.08±5.39 years (Figure 1 and Table 1).

The subjects had no significant difference in terms of basic characteristics. The mean dietary intake of participants had no significant change among groups during the study (Table 2).

Findings demonstrated the glycemic indices of AG and EG groups were significantly decreased including insulin (p=0.003 and p=0.001, respectively), HOMA-IR (p=0.01, p=0.001, respectively), and HOMA-B (p=0.002, p=0.001, respectively), but there were no significant changes in levels of serum glucose in all study groups (p>0.05) (Table 3).

Regarding apolipoproteins, the results in all groups demonstrated that ApoB was decreased significantly at the end of study compared to beginning values in AG and also EG (P =0.006 and P =0.03, respectively). Also, ApoB to ApoA1 ratio significantly decreased in both groups of AG and EG at the end of the study compared to the initial values (P =0.016 and P =0.04, respectively). Also, the mean serum levels of ApoA1 were increased at the end of the study compared to baseline values in both AG (p=0.08) and EG (p=0.05) groups (Table 3).

According to the results of ANOVA/Tukey, there was a significant difference of the insulin (p=0.04) and HOMA-B (p=0.01) changes between the AG and EG with PG, but there was no significant difference between the AG and EG. In terms of HOMA-IR (p=0.03), the differences among AG with EG and PG groups were significant, while there was no significant difference between EG and PG. There was also a significant difference in ApoB (p=0.02) among the AG group with the EG and PG, while there was no significant difference in ApoB between the EG and PG groups. Also for ApoB/ApoA1 ratio (p=0.03), there were significant differences among EG group with two groups of AG and PG, but there was no significant difference between AG and PG (Table 3).

Also, the serum levels of these variables after the intervention had statistically significant differences among the three groups as shown by using the ANCOVA test, and by omitting the confounding effect of the insulin, glucose, ApoA1, ApoB

and age before intervention (P < 0.001) (Table 4).

Discussion

In the present study, daily supplementation of aqueous and ethanolic Rheum ribes extract during 6 weeks produced significant changes on glycemic indices and apolipoproteins in patients with DMT2. The results indicate that the serum levels of Insulin and HOMA-B index were decreased in both groups of AG and EG; Moreover, the serum levels of ApoB were decreased and the serum levels of ApoA1 increased in both AG and EG groups while there were no significant changes in the control group.

The mean dietary intake among groups had no significant differences, so dietary intake could not be a confounding factor for the results of study.

During recent years, the prevalence of DMT2 has increased (2). DMT2 is one of the risk factors for developing chronic diseases including cardiovascular disease, so there is great attention to glycemic and lipid profile in patients with DMT2 (19).

Recently the effect of herbal medicine on progress and complications of DMT2 has been noticed to improve the adverse effects of chronic diseases (2). Rheum ribes is one of the wild rhubarb species that belongs to Polygonaceae family and grows in Anatolia, Iraq, and Iran. Traditionally, some kinds of Rheum ribes has been used by local people for treatment of diabetes. The other beneficial effects of Rheum ribes have been recently reported (20). Although, there are several scientific reports on the anti-diabetic effect of Rheum ribes in streptozotocin-induced diabetic rats, to our knowledge the present study was the first designed to evaluate the possible anti-diabetic effects of Rheum ribes in patients with DMT2.

In the present study, findings reported that Rheum ribes extracts significantly lead to lower serum levels of insulin, glucose and lower insulin resistance, and also, this supplementation leads to improve serum apolipoproteins. In one study, Hamzeh et al. mentioned that daily oral intake of hydroalcoholic Rheum ribes extract (150 mg/kg) for 4 weeks in alloxan monohydrate induced diabetic rats showed significantly lower serum levels of glucose (12). In another study which has been performed by Fallahhuseini et al., the evaluation of the effect of Rheum ribe supplementation in hyperlipidemic patients with DMT2 showed a reduction in the levels of glucose, LDL and total cholesterol (21). In the study by Chen et al, the supplementation of rhubarb resulted in a significant decrease of serum levels of glucose and insulin, and this supplementation leads to more insulin sensitivity (22). In an animal trial by Naqishbandi et al, the results were similar to the above studies, such a way that in the healthy mice, the serum levels of glucose decreased after receiving a single dose of Rheum ribe extract (23). These studies result was consistent with our result.

One study reported that rhubarb polyphenols such as rhaponticin increased insulin-stimulated glucose uptake as well as pioglitazone in adipocytes, suggesting that rhubarb active metabolites may enhance insulin action via activating the peroxisome proliferator-activated receptor-γ (PPAR-γ) (24). Desoxy form of Rhaponticin acts as a pro-drug and has an inhibitory action on glucose uptake of the small intestine and glucose reabsorption of renal tubular (25). Rhubarb may activate PPAR-γ coactivator-1α (PGC-1α), 5' AMP-activated protein kinase (AMPK), and Sirtuin 1 (SIRT1), which was proposed to be a potential target for developing therapeutic drugs for the treatment of DMT2 (26, 27).

In this study, we investigate the effect of Rheum ribes on apolipoproteins and we found that rhubarb and its active metabolites can attenuate the serum levels of ApoB. ApoA1 is the major apolipoprotein of HDL and it is crucial in transferring excess cholesterol from tissues to the liver. ApoB and ApoA1 have opposing effects on atherogenic risk, and ApoB/ApoA1 ratio seems very effective in characterizing the cardiovascular disease risk stronger than any other lipid ratio (28, 29).

Also, there is increasing evidence indicating that ApoB, ApoA1, and ApoB/ApoA1 ratio are powerful markers for cardiovascular disease. The previous studies showed that the high concentrations of ApoB and ApoB/ApoA1 ratio were significantly associated with a higher risk of cardiovascular disease. These apolipoproteins are also a useful indicator of lipid-lowering therapies (30).

Insulin resistance leads to the development of hyperinsulinemia, which can also cause pancreatic beta cells to be burned and lead to DMT2. In addition, increased lipid flux along with insulin resistance results in overproduction of atherogenic ApoB containing apolipoproteins (VLDL, LDL and moderate lipoprotein). High plasma levels of ApoB result in dysfunction of white adipose tissue and type 2 diabetes. Dysfunction in white adipose tissue may increase the risk of DMT2 (31). Since ApoB is directly linked to the risk of DMT2 and Rheum ribes had been an improving effect on glycemic indices as well as apolipoproteins in present study, it is likely to have a protective effect on type 2 diabetes.

Previously, it was shown that stilbenes from the rhizome of Rheum ribes including rhapontigenin, desoxyrhapontigenin, rhaponticin, desoxyrhaponticin, piceatannol, and resveratrol indicated antioxidant activity (32). One of the insulin-sensitizing and improve in lipid profile maybe refer to the antioxidant activity of Rheum ribes which can affect insulin secretory cells function and lipid oxidation.

In our knowledge, this study was the first study that compared the aqueous and alcoholic extract with each other and with a control group in diabetic patients. But, some limitations apply to this study, such as the short intervention period and small population study.

It appeared that further investigations were required for evaluating the effect of the different doses of the supplement of Rheum ribes on above mentioned and other factors in larger population of DMT2 patients and, for longer period of time, the effect of the supplement on the aforementioned factors may be more clearly indicated in the long term, or a better effect may be observed by the lower doses of herbal medicine.

Conclusion

Rheum ribes reverses metabolic impairments via insulin resistance reduction and insulin sensitivity elevation, improve apolipoproteins level in patients with DMT2. Thus it can be considered as a beneficial therapeutic approach for prevention and also treatment of diabetic complications; however, due to limited of scientific reports in this field, further studies are warranted.

Abbreviations

DMT2: type 2 diabetes mellitus; BMI: body mass index; HbA1c: hemoglobin A1c; AG: aqueous extract; EG: ethanolic extract; PG: placebo group; HOMA: homeostatic model assessment; ApoA1: apolipoprotein A-I; ApoB: apolipoprotein B; PPAR-γ: peroxisome proliferator-activated receptor-γ; PGC-1α: PPAR-γ coactivator-1α; AMPK: 5' AMP-activated protein kinase; SIRT1: Sirtuin 1.

Declarations

Ethics approval and consent to participate

The Ethical Committee of Iran University of Medical Sciences approved the study protocol, and was registered on the Iranian Registry of Clinical Trials website (http://www. irct.ir, identifier: IRCT201410142709N31). Written informed consent was received from all participants before clinical trial enrollment.

Consent for publication

A written consent was gathered from participants to publish the information and data.

Availability of data and materials

The datasets of the current study will be made available from the corresponding author on reasonable request.

Competing interests

The authors declare that there is no conflict of interest.

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Authors' contributions

F.S. was the major contributor of the manuscript, designed the research project and agreed for all aspects of the work. A.G., S.J.K., and G.H. collected and interpreted the data R.S.S. performed the statistical analysis. S.J.K, S.H, S.S, M.K, A.H and I.H made substantial contributions to the data interpretation and wrote the manuscript. All authors read and approved the final version of manuscript

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Tables

Table 1. General characteristics of participants

Variable	AG (n=20)	EG (n=20)	PG (n=20)	p-value [a]
Number, men/women	20, 12/8	20, 13/7	20, 10/10	0.15
Age (years)	53.35±5.58	53.70±4.11	55.20±4.44	0.94
Weight (Kg)	76.95±4.07	76.25±7.36	73.80±26.73	0.36
BMI (Kg/m^2)	27.14±2.72	25.51±1.90	26.73±2.11	0.29

AG = aqueous extract group; EG = ethanolic extract group; PG = placebo group.

a Obtained from ANOVA test.

Table 2. Dietary intakes of study participants throughout the study.

Variable	AG (n=20)				EG (n=20)				PG (n=20)				p-value [b]
	Week 0	Week 3	Week 6	p-value [a]	Week 0	Week 3	Week 6	p-value [a]	Week 0	Week 3	Week 6	p-value [a]	
Energy (Kcal)	2214.00±426.61	2202.00±389.31	2182.00±408.01	0.06	2108.50±381.81	2130.50±298.05	2127.61±314.21	0.15	2134.50±293.73	2109.50±269.41	2124.31±124.58	0.09	0.18
Carbohydrate (g/d)	317.15±46.33	303.40±37.80	323.00±29.70	0.86	297.85±44.93	298.50±45.38	302.54±39.56	0.57	300.00±41.31	309.60±40.95	325.18±57.69	0.74	0.28
Protein (g/d)	72.30±34.76	71.65±28.98	69.95±27.12	0.38	65.35±29.68	65.20±28.04	64.91±31.10	0.20	60.85±18.64	59.40±18.27	61.25±17.20	0.06	0.13
Total fat (g/d)	81.60±17.99	78.25±14.25	85.81±18.17	0.45	78.60±14.60	79.45±9.19	79.14±10.09	0.85	83.85±9.10	81.30±14.25	84.00±10.81	0.14	0.61
SAFA (g/d)	22.98±6.34	19.34±3.91	21.47±2.11	0.34	20.05±5.06	19.88±5.11	19.93±6.47	0.75	20.00±4.12	22.29±5.44	21.94±4.85	0.17	0.82
MUFA (g/d)	19.75±4.33	18.33±4.57	19.81±5.15	0.98	17.73±3.63	15.65±1.65	16.76±2.95	0.45	16.08±2.38	18.72±4.29	18.66±4.62	0.25	0.74
PUFA (g/d)	11.10±2.42	11.11±1.34	11.08±2.07	0.27	11.42±2.06	11.27±1.63	11.64±1.80	0.06	11.39±1.67	11.57±2.23	11.44±2.17	0.09	0.19
Vitamin A (mg)	415.25±66.74	386.15±51.46	367.27±45.34	0.07	388.65±67.92	417.45±61.85	397.27±55.24	0.52	390.85±48.69	397.85±62.35	400.21±51.47	0.096	0.36
Vitamin E (mg)	4.41±0.89	4.89±0.80	4.47±0.99	0.63	4.61±0.93	4.91±0.81	4.68±0.97	0.85	4.89±0.80	4.67±0.93	4.80±0.12	0.08	0.91
Vitamin D (µg)	5.15±0.51	5.00±0.62	5.08±0.81	0.58	4.86±0.72	5.08±0.62	5.05±0.24	0.95	4.81±0.70	4.82±0.67	4.20±0.90	0.25	0.82
Vitamin C (mg)	17.75±3.70	16.72±3.17	17.28±1.18	0.45	16.83±3.33	17.59±3.14	17.04±2.67	0.14	16.84±3.03	16.19±2.76	16.96±2.55	0.076	0.06
Calcium (mg)	1056.30±167.68	1019.00±127.36	1087.01±133.36	0.08	948.35±240.95	901.95±177.93	901.95±185.76	0.28	892.20±168.80	917.50±174.85	908.63±185.25	0.59	0.98
Iron (mg)	13.56±2.69	10.90±2.10	10.74±10.54	0.06	13.01±2.60	13.32±2.83	13.05±2.21	0.92	10.85±2.38	12.60±2.49	11.91±1.24	0.25	0.76
Selenium (µg)	0.05±0.01	0.04±0.01	0.04±0.18	0.09	0.04±0.01	0.04±0.00	0.04±0.00	0.63	0.04±0.00	0.04±0.00	0.04±0.01	0.39	0.49
Zinc (µg)	7.78±1.15	7.31±1.09	7.97±1.85	0.12	6.76±1.62	7.26±1.02	7.04±1.51	0.45	6.01±1.00	6.06±1.09	6.04±1.02	0.81	0.09
Fiber (g/d)	13.70±2.60	14.37±2.50	1.98±1.84	0.74	14.19±3.64	14.48±3.23	14.20±3.17	0.25	15.19±2.80	15.17±3.18	15.22±3.84	0.79	0.70

Data are means ± standard deviations. AG = aqueous extract group; EG = ethanolic extract group; PG = placebo group; SFA = Saturated fatty acid; PUFA = polyunsaturated fatty acid; MUFA = monounsaturated fatty acid.

a p-value for repeated measures ANOVA performed to assess variations in dietary intakes across periods.

b Obtained from ANOVA test on dietary intakes at week 6.

Table 3. Effect of aqueous and ethanolic extracts of rheum ribe L. intake on glycemic indices and apoproteins

le	AG (n=20)			EG (n=20)			PG (n=20)			P-value[b]
	Before	After	P-value[a]	Before	After	P-value[a]	Before	After	P-value[a]	
e(mg/dL)	145.75±29.95	140.85±21.85	0.09	139.43±26.82	136.56±25.39	0.11	131.34±23.67	128.65±22.82	0.11	0.78
(µIU/ml)	7.04±5.14	6.24±4.35	0.003*	8.09±4.14	6.63±3.59	0.001*	8.13±4.32	8.45±3.97	0.13	0.04
-IR	2.17±1.50	1.85±1.30	0.01¥	2.03±1.14	2.09±0.97	0.001	2.20±1.31	2.22±1.19	0.11	0.03
-B	1.36±0.91	1.05±0.74	0.002§	1.40±0.67	1.17±0.70	0.001§	1.27±0.68	1.21±0.59	0.81	0.01
(mg/dl)	147.40±21.53	151.90±21.53	0.08	115.05±36.36	122.40±23.12	0.05	154.05±28.56	142.65±24.84	0.09	0.13
mg/dl)	92.95±29.39	85.60±20.30	0.006	89.70±24.42	88.55±14.53	0.03	89.91±17.77	93.50±19.88	0.35	0.02
ApoA1	0.58±0.14	0.56±0.14	0.016	0.80±0.14	0.74±0.17	0.04‡	0.59±0.13	0.58±0.09	0.08	0.03

All values are means ± SD. AG = aqueous extract group; EG = ethanolic extract group; PG = placebo group; HOMA-IR = homeostasis model of assessment-insulin resistance; HOMAB = homeostatic model assessment-Beta cell function; ApoB = Apolipoprotein-B; ApoA1 = Apolipoprotein AI.

a Obtained from paired T-Test.

b Obtained from ANOVA test.

*Significant differences with PG according to ANOVA/Tukey. ¥ Significant differences with EG and PG according to ANOVA/Tukey. § Significant differences with PG according to ANOVA/Tukey. Significant differences with EG and PG according to ANOVA/Tukey. ‡ Significant differences with AG and PG according to ANOVA/Tukey.

Table 4. Adjusted changes in metabolic variables in study participants.

Variable	AG (n=20)	EG (n=20)	PG (n=20)	P-value [a]
Insulin (µIU/ml)				
Model 1[b]	-0.82±0.98	-1.48±0.48	0.41±0.85	0.03
Model 2[c]	-0.84±0.98	-1.48±0.48	0.40±0.85	0.03
HOMA-IR				
Model 1	-0.35±0.14	0.08±0.09	0.02±014	0.03
Model 2	-0.36±0.14	0.09±0.09	0.05±013	0.03
HOMA-B				
Model 1	-0.38±0.23	-0.26±0.08	-0.05±0.12	0.01
Model 2	-0.39±0.23	-0.25±0.08	-0.04±0.11	0.01
APO-B (mg/dl)				
Model 1	-12.19±9.74	-1.88±10.21	-3.98±3.45	0.03
Model 2	-12.11±9.74	-1.82±10.21	-3.90±3.46	0.03
APO-B/APO-AI				
Model 1	-0.02±0.01	0.71±0.02	-0.01±0.03	0.04
Model 2	-0.04±0.01	0.75±0.02	-0.02±0.03	0.04

All values are means ± standard errors. AG = aqueous extract group; EG = ethanolic extract group; PG = placebo group;HOMA-IR = homeostasis model of assessment-insulin resistance; HOMAB = homeostatic model assessment-Beta cell function; APO-B = Apoprotein-B; APO-AI = Apoprotein AI.

a Obtained from ANCOVA. b Adjusted for baseline values. c Further adjusted for Model 1 + age.

Figures

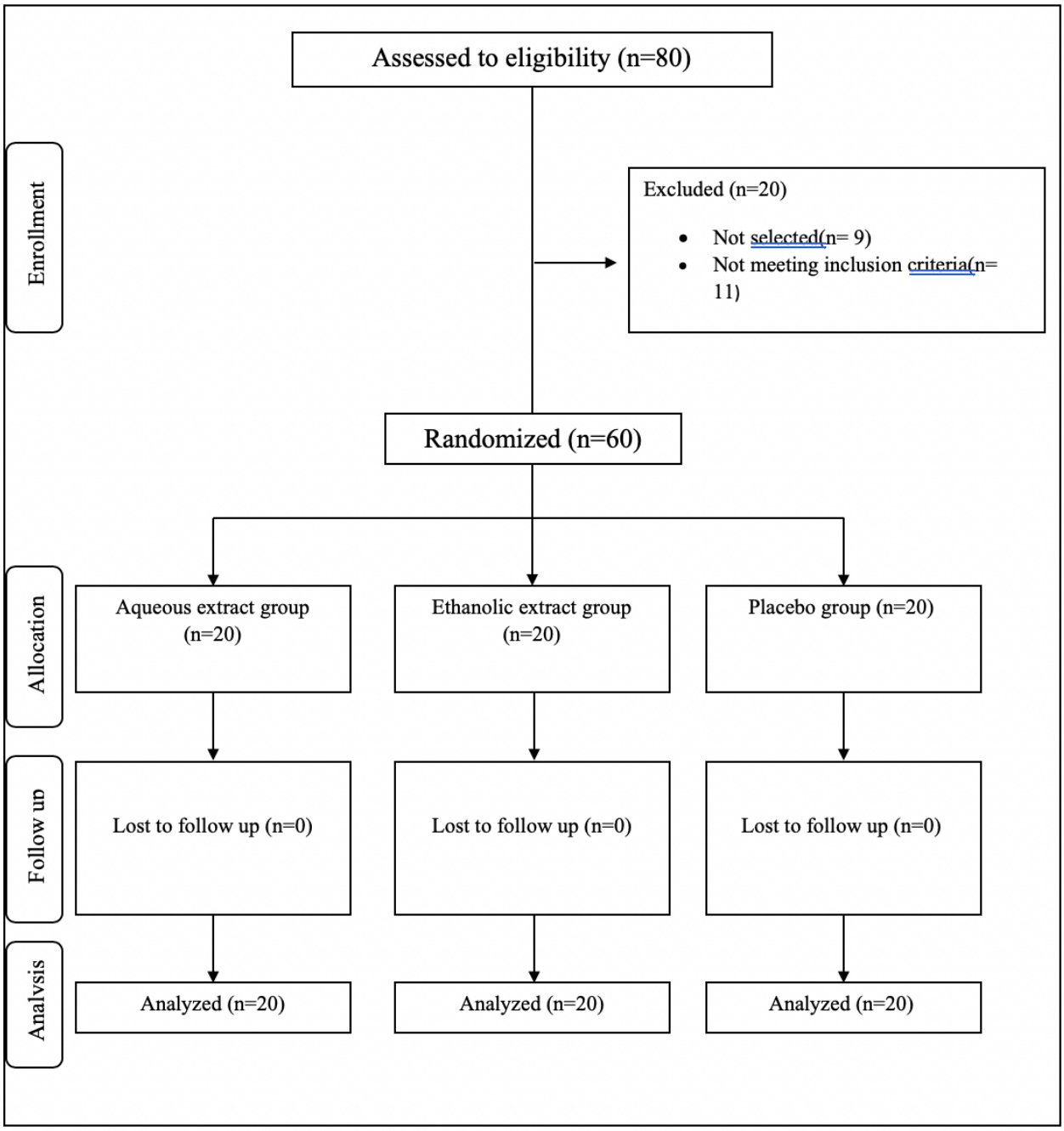

Figure 1

Summary of patient flow diagram