The Effects of Rutin Flavonoid Supplementation on Brain White Matter and Gray Matter Abnormalities in Type 2 Diabetes Mellitus Patients: A Diffusion Tensor Imaging -Based Study Protocol for a Double-Blind, Randomized Controlled Trial

Ali Reza Eftekhari Moghadam
Ahvaz Jondishapour University of Medical Sciences Faculty of Paramedical Sciences
Ahmad Zare Javid
Ahvaz Jondishapour University of Medical Sciences Faculty of Paramedical Sciences
Jafar Fatahi Asl
Ahvaz Jondishapour University of Medical Sciences Faculty of Paramedical Sciences
Leila Moradi
Ahvaz Jondishapour University of Medical Sciences Faculty of Medicine
Abolhasan Rezaeyan
Iran University of Medical Sciences: Tehran University of Medical Sciences
Hossein Bavi Behbahani
Ahvaz Jondishapour University of Medical Sciences Faculty of Paramedical Sciences
Hadi Bazyar (hadibazyar2015@gmail.com)
Ahvaz Jondishapour University of Medical Sciences Faculty of Paramedical Sciences
https://orcid.org/0000-0002-1627-7122

Study protocol

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Abstract

Background: Brain microstructural changes in white matter (WM) and gray matter (GM) of type 2 diabetes mellitus (T2DM) patients can be explained using Diffusion Tensor Imaging (DTI) method. Increased oxidative stress has been recognized the key factor in T2DM induce neural damage. Rutin flavonoid plays protective roles in several oxidative stress-mediated neurodegenerative disorders. So, the effects of rutin on WM and GM continuous changes in T2DM patients requires to be more investigated.

Method: We will conduct a 3 months, double-blind, randomized controlled clinical trial to examine the effects of rutin supplementation on WM and GM changes in 26 T2DM cases. The intervention group (n=13) and the control group (n=13) will receive one tablet of 1 g rutin/day and one tablet of 1 g placebo/day after meals, respectively. Before and after the intervention, DTI will perform at 1.5 Tesla, followed by an analysis employing tract-based spatial statistics (TBSS) to explore the changes in fractional anisotropy (FA), mean diffusivity (MD), axial diffusivity (λ1), and radial diffusivity (λ23) among the rutin and placebo treated groups. A receiver operating characteristic (ROC) test will use to evaluate the performance of DTI criteria for cutting off the two T2DM groups.

Discussion: We will demonstrate the diverse influences of rutin supplementation on WM and GM integrity on T2DM patients. Since, rutin may regulate neurotoxicity, oxidative stress, inflammatory reaction and nitric oxide production in nervous system; for the first time, the effects of rutin on WM and GM alterations will be evaluated by DTI in T2DM patients.

Trial registration: Iran Clinical Trials Registry, registration number: IRCT20151128025274N6. Registered on 10 January 2021, https://fa.irct.ir/user/trial/53283/view

Introduction

Type 2 diabetes mellitus (T2DM) is a prevalent disease and a serious public health problem that affects over 360 million people worldwide and predicted to increase to 693 million cases by 2045 [1]. T2DM is associated with negative outcomes, such as cardiovascular disease, cancer and cerebral disorders [2, 3]. T2DM, which comprise 90-95% of cases, is identified by hyperglycemia and obesity-related insulin resistance [4, 5]. Hyperglycemia plays a critical role in the development and progression of T2DM complications with various mechanisms including increased oxidative stress, reduced nitric oxide bioavailability, and glucose autoxidation [6, 7]. The sustained hyperglycemia can influence many organs such as kidney, eyes and nervous system. Brain damage caused by diabetes has interested extended consideration in recent decade [8]. Patients with T2DM are at an extended possibility of stroke, dementia, white matter lesions and cognitive impairment [9-11]. Moreover, white matter volume and integrity loss, weaker organization of white matter networks are another T2DM associated complication [12, 13]. In another survey by Zhang et al., they reported decreased total gray matter (GM) and middle temporal cortex volume in T2DM patients [14]. Neuroimaging methods have confirmed to be informative for studying the diabetic brain. Diffusion Tensor Imaging (DTI) technique is a high sensitive noninvasive

Magnetic Resonance Imaging (MRI) sequence which can detect white matter (WM) impairment. DTI specific parameters, comprising fractional anisotropy (FA) and mean diffusivity (MD), are associated to microstructural alterations in WM. Increased MD or decreased FA values indicate damage to WM microstructures and neurological disorders, while detection of such changes difficult using other conventional MRI sequences [15]. DTI parameters can be utilized to identify T2DM patients with and without neurological symptoms before clinical diagnosis [16]. In a tract based spatial statistics (TBSS) study, Tan et al. found that the damaged WM regions in T2DM patients were identical to those with Alzheimer's disease, which aided to better explain the neuropathological process of T2DM [17]. In a study by Hoogenboom et al., decreased FA in the cingulum bundle and uncinate fasciculus was observed in middle-aged patients with T2DM [18]. Other investigations demonstrated reduced FA in the frontal lobe and increased MD in bilateral hemisphere tracts of T2DM patients [19, 20]. All these microstructural brain abnormalities were proposed to be the potential imaging biomarkers for early detection of neurological symptoms in T2DM patients. However, neuroimaging findings could be determined as a suitable time-window for intervening T2DM patients in the preclinical stage to prevent occurrence of irreversible nervous system damage.

Misunderstanding of pathophysiological mechanisms of T2DM induced neurological problems can hinder the process of preventive approaches. T2DM may be also a risk factor for cerebral inflammation, possibly by enhancing the production of pro-inflammatory cytokines (e.g. TNF-α and IL-1β). These cytokines lead to further cytotoxicity and neuronal cell dysfunction [21, 22]. Therefore, inhibiting cytotoxicity, reducing oxidative stress, and decreasing produce of pro-inflammatory cytokines are reasonable therapeutics for management of diabetic brain injury.

Flavonoids are a family of phenolic compounds with unique antioxidant activities and neuroprotective properties which play an essential role in oxidative stress-mediated conditions, including diabetes [23, 24]. Among flavonoids, the most usual is bioflavonoid rutin (vitamin P) (3,30,40,5,7-pentahydroxyflavone-3-rhamnoglucoside), the primary glycoside (a 3-orhamnoglucoside) form of quercetin that is found in food including onions, apples, tea, and red wine [25]. Flavonoids have been found influencing various aspects of brain function, including cerebrovascular blood flow and synaptic plasticity [26]. Evidence shows that flavonoids have an ability to interact with neuronal receptors and kinase signaling pathways which are key to neuronal activation, communication and synaptic strengthening [27, 28].

Hypothesis and aims

The aim of the present investigation is to examine the effects of rutin oral supplementation on white matter abnormalities and gray matter volume using DTI, on adults with T2DM. So, we hypothesize it that 3 months supplementation with rutin will improve measures of examination.

Methods

Study Design and participants

We will be performed a double-blind, parallel-group, randomized controlled clinical trial. In this study, patients will refer to the endocrinology and metabolism clinic of Golestan Hospital of Ahvaz Jundishapur University of Medical Sciences in Ahvaz, Iran from January 2021. According to the inclusion criteria, 26 patients will be included in the study. These patients will be randomly allocated into intervention (n=13) and control (n=13) groups. Then, they will be referred to Auxin Imaging Center for MRI of the brain by DTI method. Figure 1 illustrates the Flow diagram of the research.

Randomization and blinding

Assignment of patients in each of the study groups (supplements or placebo) will be done by random allocation software using classified randomized blocking method (4 blocks). In addition, in order to reduction selection bias error, allocation concealment will be used. This will be done by assigning unit codes to each patient's tablets. In fact, each patient will receive a can containing code A or B, and eventually 13 patients will receive a can containing code A and 13 patients will receive a can containing code B**.** In the present study, the researcher, and patients will be blinded to the studying groups (double-blind). Before starting the study, the cans containing the respective tablets will be coded by a person other than the researcher (this person will not aware of the details of the research) to A and B, so that the type of received tablets in each group will be blinded for researcher.

Inclusion and exclusion criteria

Inclusion criteria will be included patients aged 18 to 60 years old; body mass index (BMI) range of 18.5 to 35 kg/m^2 and glycosylated hemoglobin (HbA1c) between 6.5 to 11%. The patients with any of the following criteria will be excluded from the study: history of severe traumatic brain injury (sTBI), having a history of brain tumor and CNS radiation therapy or surgery, pregnancy and lactation; acute or chronic renal failure, acute or chronic hepatic failure, uremia hemodialysis, thyroid disorders, anemia, modifications in diet during the study period, unwilling to continue, diabetic patients taking insulin, smokers, consumption of other antioxidant and dietary supplements in the last 3 months, anti-inflammatory and immunosuppressive medications. According to the guideline, people with hemoglobin A1c (HbA1c) ≥ 6.5%, fasting plasma glucose (FPG) ≥ 126 mg/dl, or 2-hour plasma glucose (2hPG) ≥ 200 mg/dL will be considered as diabetic patients [29].

Intervention protocol

The intervention group will receive one tablet of 1 g rutin/day (made by Solgar company, USA, containing 500 mg of pure rutin and 500 mg dicalcium phosphate, microcrystalline cellulose, plant cellulose, stearic acid, stearic magnesium, silica, glycerin) after meals for 3 months. The control group will receive one tablet of 1 g placebo/day (compounds similar to supplement except for rutin) after meals for 3 months. Also, placebo and supplement tablets will be similar in terms of color, shape, size and taste. In addition, cans containing the supplement and placebo will be quite similar. To monitor adoption, patients will be requested to note the time and date of supplement intake. To check that the patients consume the supplement, they will be contacted every 14 days by a dietitian, or if it was not available to call them, they

would be traced via Short Message System. Patients will be advised not to change their diet and physical activity during the study. Counting of remaining tablets will be conducted to assess compliance of patients. Those who have not taken more than 20% of the tablets will be excluded from the study. Based on the Standard Protocol Items; Recommendations for Interventional Trials (SPIRIT) Figure, in figure 2, it has been shown the schedule for enrollment, intervention and assessment

Ethics and trial registration

At the beginning of the study, informed consent forms will be taken from all patients by researcher. The protocol was approved by the Ethics Committee of the Ahvaz Jundishapur University of Medical Sciences that is under Declaration of Helsinki (approval number: IR.AJUMS.REC.1399.777). All collected data will be held private. This trial was registered at the Iranian Registry of Clinical Trials (registration number: IRCT20151128025274N6).

Assessment of dietary intake, anthropometric indices, physical activity, and medications

A demographic questionnaire will be given at the beginning of the research. Dietary intake will be checked by 3 days' food record program (two weekdays and one weekend day) at the onset and end of the survey. Participants will be explained about the food recording by an educated dietician. Dietary intake data will be interpreted by Nut IV software (the Hearst Corporation, San Bruno, CA). The same dietitian will measure anthropometric variables such as body weight, height, body mass index (BMI), and waist and hip circumference (WC, HC). Body weight will be measured with the accuracy of 100 gr using a Seca scale at the baseline and end of the research. Height will be evaluated in a relaxed position by a Seca stadiometer with an accuracy of 0.5 cm. Then, BMI will be computed as body weight (kg) divided by the square of height (m), the beginning and end of the study. WC and HC will be measured using a tape meter at the midpoint between the lowest rib and iliac crest, at the end of a normal expiration and distance around the largest part of hips (the widest part of buttocks) to the nearest 0.5 cm. To gain physical activity levels of participants, the International Physical Activity Questionnaire (IPAQ) will be given at the baseline, and the end of the survey in a consultation, and the results will be illustrated as "high," "moderate," and "low" activity. The Persian version of the brief form IPAQ has been approved by Dashti et al. (Cronbach's alpha=0.7 and test–retest reliability coefficient=0.9) [30].

In this study, patients who take only tablet will be included in the study. Patients' medications will be checked at the beginning and end of the study. At the baseline, medications between the two groups will be the same in terms of the type and dosage.

Measurements

All T2DM patients will have undergone brain imaging before and after the rutin supplementation. DTI will have performed at 1.5 Tesla MRI machine using a commercial 24-channel head coil (MAGNETOM Aera, Siemens Healthcare, Erlangen, Germany) followed by an analysis utilizing tract-based statistics to study the differences in fractional anisotropy (FA), mean diffusivity (MD), axial diffusivity (λ1), and radial

diffusivity (λ23) among the groups [16]. The MRI protocols will be implemented corresponding to the following criteria as previously reported by Xiong et al., [16]: Applying axial T2-FLAIR (TR/TE/TI = 8400/160/2100 ms, slice thickness = 5 mm, slice spacing = 1.5 mm, matrix size = 256×256, FOV = 24.0×24.0 cm2, and NEX = 1) and sagittal T1-weighted three-dimensional brain volume imaging sequences (TR/TE/TI = 8.2/3.2/450 ms, flip angle = 12°, slice thickness = 1 mm, matrix size = 256×256×160, FOV = 25.6×25.6 cm2, and NEX =1), high-resolution anatomical images will obtained to exclude possible lesions defined in the exclusion criteria [16]. Following anatomic imaging, we will obtain DTI data in the axial plane utilizing a single-shot diffusion-weighted echo planar imaging sequence with the following parameters: TR/TE = 8500/66.3 ms, FOV = 25.6×25.6 cm2, matrix size = 128×128, slice thickness = 2 mm, number of slices = 70, number of diffusion gradient directions = 64, b-value = 1000 s/mm2, number of images at b-value of 0 s/mm2 = 5, acceleration factor = 2, and scan time = 9 minutes and 55 seconds [16]. The DTI will have processed utilizing the Functional Magnetic Resonance Imaging of the Brain (FMRIB) Software Library (http://www.fmrib.ox.au.uk/fsl), or FSL [31]. Voxel-wise statistical evaluation of the images will have carried out adopting tract-based spatial statistics (TBSS) with the following steps [16]. First, brain will extract employing the brain extraction tool, and an FSL “eddy” tool will implement as a preventive procedure to diminish inconsistent image distortion. After developing the FA maps using the FMRIB diffusion toolbox, the images from all patients will adjust to an FA standard template through a nonlinear co-registration. The aligned FA maps will again average to generate a group mean image, which applied to make an FA skeleton highlighting the tracts common to the entire group. The resulting skeletonized FA maps will then provide into a Voxel-wise group-level analysis [32]. Besides FA, diffusivity maps based on MD, axial diffusivity (λ1; the main eigenvalue), and radial diffusivity (λ23; the mean of the two remaining eigenvalues) will also develop adopting the identical aforementioned steps. Using an FSL permutation experiment (FSL Randomize Tool with 500 permutations), FA, MD, λ1, and λ23 will be assessed for differences between the means of the T2DM placebo and rutin treated groups. A significance level of $p < 0.05$ will use for each of the four DTI parameters (FA, MD, λ1, and λ23) to indicate a difference between the patient groups. ROI-based Quantitative Analysis: The Johns Hopkins University (JHU) WM tractography atlas in FSL will utilize as a standard for WM parcellation [33]. The whole WM will parcel into 48 ROIs applying the 1mm JHU-ICBM-labels [11, 34]. Distinct fiber tracts investigated to be related to T2DM in early investigations will choose in the telencephalon which include: corticospinal tract, inferior fronto-occipital tract, superior longitudinal fasciculus, internal capsule, forceps major and Cingulum. MRI data of gray matter volume in auditory, visual, frontal and parietal cortex areas in rutin and placebo supplemented group will transfer to freeSurfer software version 2.5 [35], then the cortex size will be compared with each other [20].

Sample size

Given that there is no similar study to determine the sample size and according to the nature of the study and based on other similar studies that have used other flavonoids [36] and according to the statistical consultant, the sample size of 10 patients will be considered for each intervention and control groups. Considering a 30% withdrawing rate, 13 patients for each group and a total of 26 patients will be included in this study.

Statistical analysis

Normal data are reported as mean ± standard deviation and abnormal data as median (minimum, maximum) and are analyzed with the Statistical Package for the Social Sciences (SPSS) version 24 (SPSS, Chicago, IL, USA). The intention-to-treat (ITT) method will be performed to analyze the data. Qualitative data will be compared with Chi-square test. The Paired t-test (normal data) and Wilcoxon signed-rank test (abnormal data) will utilize for the analysis of differences between baseline and after the intervention in each group. The Independent t-test will use to compare quantitative variables in the two groups and Mann-Whitney will utilize if it is not normal. The Analysis of covariance (ANCOVA) test will use to determine changes confounding variables between the two rutin and control groups post-intervention after adjusting the confounding variables. A p-value < 0.05 will be considered statistically significant.

Safety, adverse effects and monitoring data

No side effects have been reported for supplementation with 500 mg of rutin per day [37]. However, this study will controlled by a Data Monitoring Committee (DMC). Furthermore, any possible side effects of supplementation will be reported to the Ethics Committee of the Ahvaz University of Medical Sciences.

Discussion

Evidences show hypothesis that the WM alteration in T2DM patients is a slow and continuous phenomenon that may not be appropriately displayed by neuropsychological test scores, but can be captured in DTI parameters [16]. Moreover, prohibiting of neural cytotoxicity, reducing oxidative stress, and decreasing the production of pro-inflammatory cytokines are reasonable therapeutics for management of diabetic brain damage and subsequent neurological impairments [24]. Flavonoids, specifically rutin, are a group of phenolic compounds with antibacterial, antiviral, anti-inflammatory, anti-allergic, antithrombotic, and neuroprotective effects [26]. However, the effect of rutin supplementation on WM tracts integrity and GM volume in adult T2DM remain obscure. This TBSS-based DTI research should define another valuable effect of rutin supplementation by which WM integrity and GM volume improve in T2DM patients.

Trial Status

Protocol version IRCT20151128025274N6. Registered on 2021-01-10. Recruitment of patients started on 2021-01-20 and will be completed approximately on 2021-07-23.

Abbreviations

Type 2 diabetes mellitus (T2DM),white matter (WM), Magnetic Resonance Imaging (MRI), gray matter (GM), Diffusion Tensor Imaging (DTI), tract-based spatial statistics (TBSS), fractional anisotropy (FA), mean diffusivity (MD), axial diffusivity (λ1), radial diffusivity (λ23), receiver operating characteristic

(ROC), body mass index (BMI), waist and hip circumference (WC, HC), glycosylated hemoglobin (HbA1c), severe traumatic brain injury (sTBI), International Physical Activity Questionnaire (IPAQ), nutritionist 4 (Nut 4).

Declarations

Availability of data and materials

The results will not be available before publishing.

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Authors' contributions

HB and AEM had the idea of the research. HB, AEM, AZJ, LM, and JFA contributed to design of study. HB, HBB, AR, and AFM will perform the study. HB and AEM prepared the first version of manuscript. AZJ and LM revised the manuscript. AEM, AR, and JFA will interpret the MRI-related results. HB and HBB will do statistical analysis. All authors have approved the final version.

Competing interests

The authors declared no conflict of interest.

Consent for publication

Not applicable.

Ethics approval and consent to participate

At the beginning of the study, informed consent forms will be taken from all patients. The protocol was approved by the Ethics Committee of the Ahvaz Jundishapur University of Medical Sciences that is under Declaration of Helsinki (approval number: IR.AJUMS.REC.1399.777).

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Figures

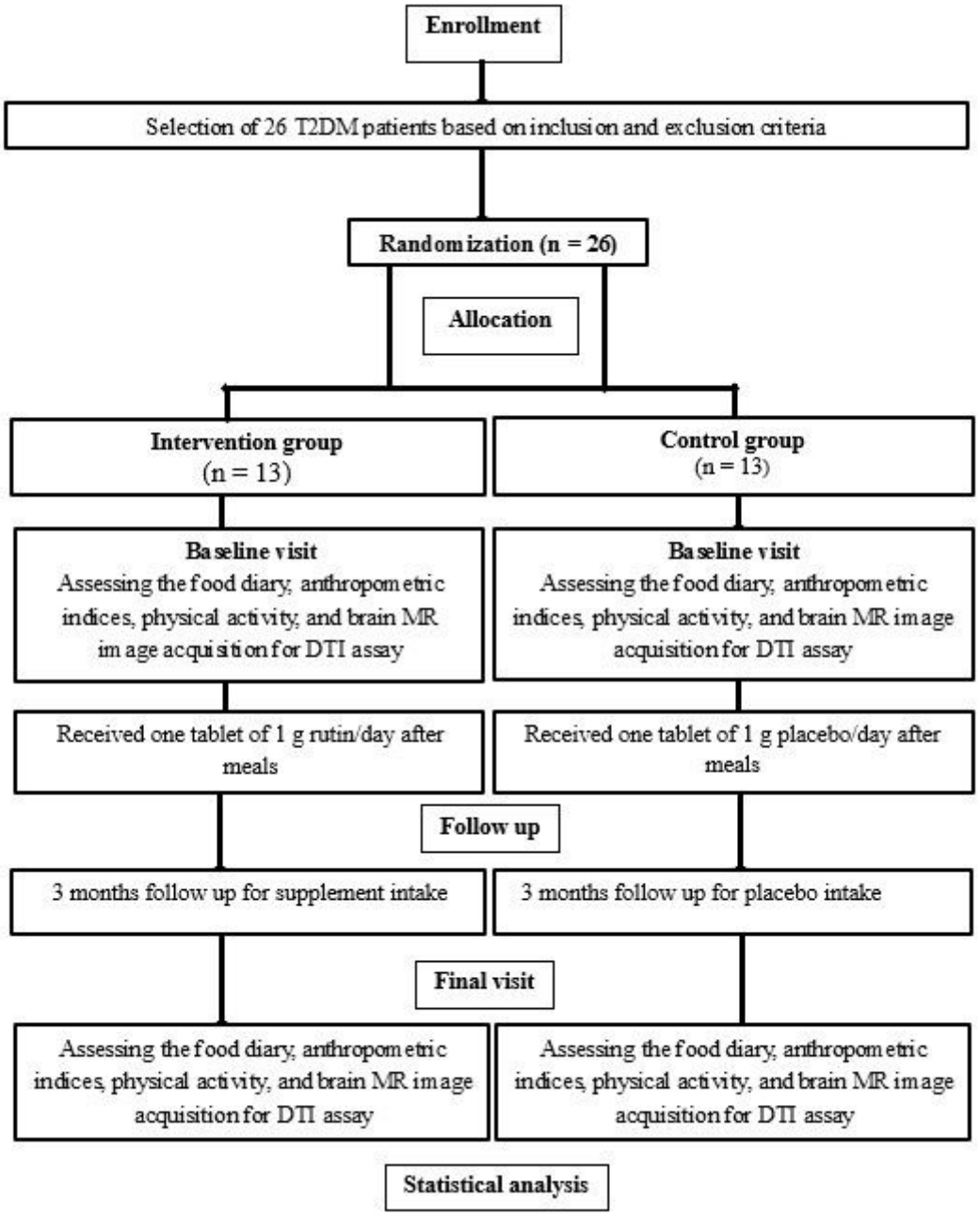

Figure 1

Flow diagram of the research

	STUDY PERIOD				
	Enrolment	Allocation	Treatment phase		Follow up
TIMEPOINT**	-week	Baseline (week 0)	Baseline (week 0)	Month 3	3 months after the baseline
ENROLMENT:					
Eligibility screen	X				
Informed consent	X				
Allocation		X			
INTERVENTIONS:					
[Rutin]			←	→	
[Placebo]			←	→	
ASSESSMENTS:					
[MRI, anthropometric indices, 3 days' food record and etc.]	X	X			
[MRI, anthropometric indices, 3 days' food record and etc.]					X

Figure 2

Schedule for enrollment, intervention and assessment based on Standard Protocol Items; Recommendations for Interventional Trials (SPIRIT) Figure

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SPIRIT2013Checklist.doc