Argyreia Nervosa Attenuates Insulin Resistance in Diabetic Gastrocnemius Muscle in Experimental Diabetic Rats: Role of Nrf2/Keap-1 Signaling Pathway

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Abstract

Introduction:

Diabetes interferes with the body's ability to metabolic sugar for energy. By far the most devastating aspect of diabetes is the long term complications it causes. Argyreia nervosa is a large climber known for its pharmacological properties which includes anti-microbial, analgesic, anti-inflammatory and various other properties.

Materials and Methods:

Fair groups of rats were taken, six rats in each group.The Group-1 rats were kept normal as such.Group-2 were diabetic rats. Group-3 as diabetic rats treated with A.nervosa.Group-4 rats were normal rats treated with A.nervosa. Then the animal is sacrificed and the gastrocnemius muscle is isolated. This is further analyzed through gene expression along with Real-time PCR.

Results and conclusion:

The control condition was normal.In diabetic condition NrF2 was reduced and Keap-1 gene was increased. The present study shows that STZ alters the glucose metabolism which was treated by A.nervosa.

Keywords: Insulin resistance, diabetes, novel method, gastrocnemius muscle , gene expression analysis , glucose metabolism, innovative technique.

1. INTRODUCTION

Argyreia nervosa , belonging to the family convolvulaceae is commonly called as elephant creeper or wooly morning glory. This plant grows near edges of lakes and river banks.The leaves of Argyreia nervosa contain β-sitosterol, 1-triacontanol and quercetin.Traditionally it is widely used for its antiviral, anti-bacterial, anti-fungal and anti inflammatory properties (1).

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Diabetes interferes with the body's ability to metabolize sugar for energy. The most devastating aspect of diabetes is the long term complications it can cause.The prevalence of diabetes in india has risen from 7.1% in 2009 to 8.9% in 2019.The most common type of diabetes is type 2 diabetes(T2D). T2D results from the inability of the body to properly use insulin (2). It is also associated with body weight and also concerned for physical inactivity (Kubota et al;2017).

Nuclear factor erythroid 2-related factor 2 (NRF2) is a transcription factor that regulates the cellular defense against toxic and oxidative insulin.(3) Keap-1 is a gene encoding protein containing KELCH-1 like domains. Thus the present study that streptozotocin can alter the glucose metabolism and assess the modulatory effects of repeated administration of A.nervosa to model T2D characteristics in Wistar rats. (4). Our team has extensive knowledge and research experience that has translate into high quality publications (5–14))(15–24)). The aim of this study is to analyze A.nervosa attenuates insulin resistance in diabetic gastrocnemius muscle in operational diabetic rats: role of NaF1/Keap-1 Signaling pathway.

2. MATERIALS AND METHODS

Chemicals Used:
The entire chemicals and reagents used in this research were of the molecular and analytical grade acquired from Sigma Chemical Company, and Sisco Research Laboratories (Mumbai, India).

Plant collection:
The species will be verified at Anna Siddha Hospital in Chennai, Tamil Nadu, using Argyreia nervosa root powder obtained from a pharmacy.

Extract preparation
The roots of Argyreia nervosa powder were soxhlet extracted with 70% ethanol. The extract was then filtered with Whatman no. 1 filter paper and the solvent evaporated at reduced pressure by using a Rotary evaporator apparatus to get a viscous mass, which was then stored at 4°C until used.

Animals
Animals were maintained as per the National Guidelines and Protocols approved by the Institutional Animal Ethics committee (BRULAC/SDCH/SIMATS/IAEC/04-2022/109). Healthy adult male Wistar albino rats of Wistar strain (150–180 days old weighing 180–200 g) were used in this study and maintained in clean polypropylene cages at the Biomedical Research Unit and Lab Animal Center (BRULAC), Saveetha Dental College & Hospitals, Saveetha Institute of Medical & Technical Sciences, Chennai – 600 077, Tamil Nadu, India, under specific humidity (65 ± 5%) and temperature (21 ± 2°) with constant 12 h light and 12 h dark schedule. The standard pellet diet (Lipton India, Mumbai, India) was provided with clean drinking water in ad libitum.

STZ induction
Diabetes was induced in rats by a single intraperitoneal administration of STZ (55 mg/kg) dissolved in 0.1 M citrate buffer, pH 4.5. 48 hours later, blood samples were collected and glucose levels were estimated to confirm the development of diabetes. The rats that showed hyperglycemia (blood glucose level > 250 mg/dl) were selected for experimental study (Shiv 2010).

Grouping of animals
Animals were grouped into 3 groups of six animals each and treated oral administration for 15 days.
Group I – Normal rats

Group II- diabetic rat

Group III - diabetic rat + oral administration of argyreia nervosa 500 mg/kg/day

Group IV - normal rat + oral administration of argyreia nervosa 500 mg/kg/day

Parameters to be studied

Fasting blood glucose (FBG)

After the overnight fasting, the blood glucose was estimated using On-Call Plus blood glucose test strips (ACON Laboratories Inc., USA). From the rat tail tip, the blood was collected and the results were expressed as mg/dl.

Oral glucose tolerance test (OGTT)

For the oral glucose tolerance test, animals fasted overnight. After giving the oral glucose load (10 ml/kg; 50% w/v) blood glucose level was estimated at various time periods (60, 120, and 180 min) by using On-Call Plus blood glucose test strips. Before giving a glucose load, the value of blood glucose is considered as 0 min value. Results were marked as mg/dl.

Fasting serum insulin

Serum insulin was assayed using ultrasensitive rat insulin ELISA kit obtained from Crystal Chem Inc (Illinois, USA). The range of detection is 0.1–64 ng/ml. The percentage cross reactivity of insulin antibody to rat insulin was 100%. The intra assay coefficient of variation was ≤10.0% and inter-assay coefficient of variation was ≤10.0%. Results were expressed as mIU/ml.

Total RNA isolation, cDNA conversion and real-time PCR

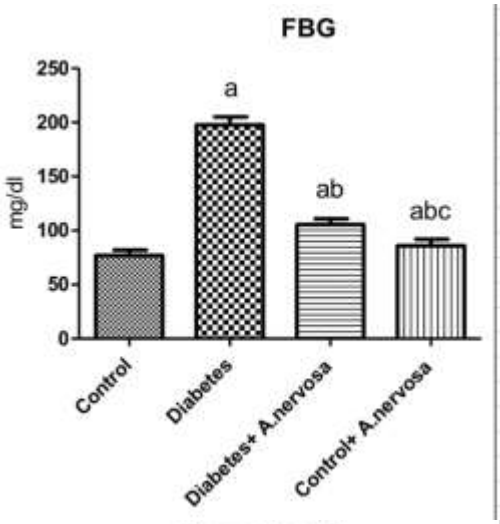
Using a TRIR kit (Total RNA Isolation Reagent Invitrogen), total RNA was isolated from control and experimental samples. In brief, to 100 mg of fresh tissue, 1 ml of TRIR was added and homogenized. The content was transferred to a microcentrifuge tube instantly and 0.2 ml of chloroform was added, vortexed for 1 min then kept at 4ө C for 5 min. Later, the contents were centrifuged at 12,000 ×g for 15 min at 4°C. The aqueous phase (upper layer) was carefully transferred to a fresh microfuge tube and an equal volume of isopropanol was added, vortexed for 15 S and placed on ice for 10 min. After centrifugation of the content at 12000 ×g for 10 min at 4°C, the supernatant was discarded and RNA pellet was washed with 1 ml of 75% ethanol by the vortex. The isolated RNA was estimated spectrometrically. The RNA concentration was expressed in micrograms (µg). By using the reverse transcriptase kit from Eurogentec (Seraing, Belgium), complementary DNA (cDNA) was synthesized from 2 µg of total RNA as stated in the manufacturer's protocol. To perform real-time PCR, the reaction mixture containing 2x reaction buffer (Takara SyBr green master mix), Forward and reverse primers of the target gene and house-keeping gene, water and β-actin (the primer sequences were listed in Table 1) in total volume of 45 µl expect the cDNA was made, mixed intensively and spun down.

In individual PCR vials, about 5 µl of control DNA for positive control, 5 µl of water for negative control and 5 µl of template cDNA for samples were taken and a reaction mixture (45 µl) were added. 40 cycles (95°C for 5 min, 95°C for 5 s, 60°C for 20 s and 72°C for 40 s) was set up for the reaction and obtained results were plotted by the PCR machine (Stratagene MX 3000 P, Agilent Technologies, 530 l, Stevens Creek Blvd, Santa Clara CA, 95051) on a graph. Relative quantification was calculated from the melt and amplification curves analysis.

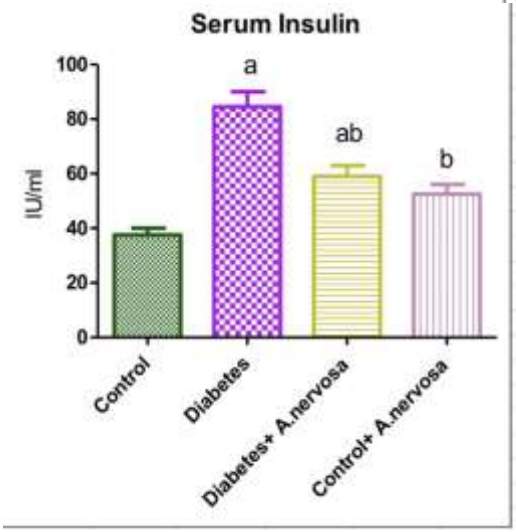
Statistical analysis

The data will be analyzed statistically and ONE-WAY- ANOVA will be used followed by Dencan’s multiple range test will be used to check statistical significance among groups. The significance will be considered at the levels of $P<0.05$.

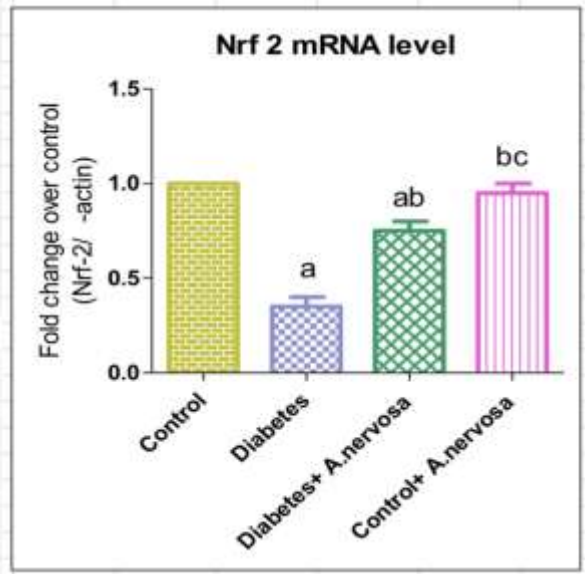
3. RESULTS AND DISCUSSION



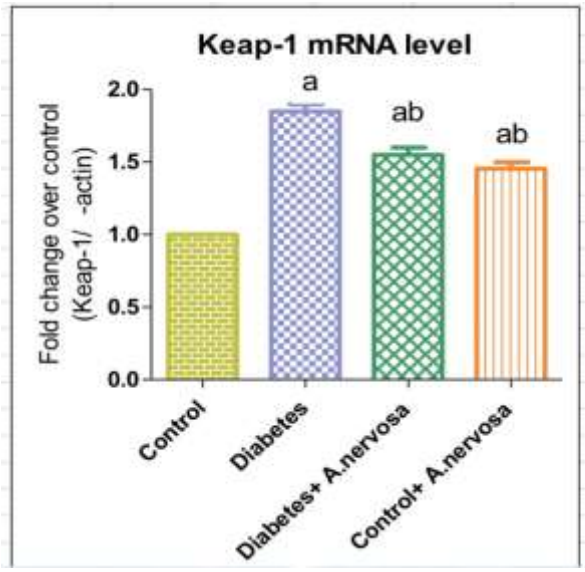
FBG				
Mean	77	197.5	105.5	86
Std. Devia	7.071	10.61	7.778	8.485
Std. Error	5	7.5	5.5	6



Serum insulin				
Mean	37.5	84.5	59	52.5
Std. Devia	3.536	7.778	5.657	4.95
Std. Error	2.5	5.5	4	3.5



Mean	1	0.35	0.75	0.95
Std. Devia	0	0.07071	0.07071	0.07071
Std. Error	0	0.05	0.05	0.05



Keap-1				
Mean	1	1.85	1.55	1.455
Std. Devia	0	0.07071	0.07071	0.06364
Std. Error	0	0.05	0.05	0.045

Insulin resistance promotes impaired fasting blood glucose levels which increases the prevalence of move atherogenic lipid profile molecules. (4,25) The consequences continue to grow into the factors and other cardiovascular diseases depending on the number of components present (26). Preclinical models of experimental rodents are updated.(Engel et al;2019).

By inhibiting the exocytosis of the auxin indolyl acetic acid and by inducing gene expression, flavonoids control plant growth. They also have a variety of additional effects on other biological cells. Their toxicity to mammalian cells, however, is minimal. Many herbal and insect preparations for medical use, such as propolis (bee's glue) and honey, which have been used since ancient times, contain flavonoids as important functional ingredients. Flavonoids should be consumed in amounts of 1-2 g per day with typical meals, particularly fruit and vegetables. Due to their demonstrated capacity to inhibit certain enzymes, mimic some hormones and neurotransmitters, and scavenge free radicals, modern licenced physicians are increasingly using pure flavonoids to treat many serious common disorders (27).

The majority of the diabetic mice received treatment with MSD, FSD, and glibenclamide saw a rise in body weight. Comparing the diabetic control group to the normal control, extracts, and glibenclamide treatment groups, the diabetic control group demonstrated a substantial (p 0.05) drop in body weight (28).

The breakdown of carbohydrates into monosaccharides for absorption is carried out by digestive enzymes for carbohydrates, primarily a-amylase and a-glucosidase . For the treatment of non-insulin diabetes, natural substances made from traditional medicinal herbs that could suppress these digestive enzymes would be helpful. It has been demonstrated that A. nervosa seed and leaf compositions have powerful anti-inflammatory and anti-diabetic effects. (29)

In the present study , STZ included group shows the increases in FBG and serum insulin when compared to the control group, whereas in the A.nervosa treated group the FBG and serum insulin levels were restored to near normal levels.

The mRNA expression levels of NrF2 was decreased and Keap-1 was increased in diabetic condition, whereas in the A.nervosa treated group these genes were

regulated compared with the control group. The present study shows that A.nervosa has the anti-diabetic activity by regulating NrF2/Keap-1. Our team has extensive knowledge and research experience that has translate into high quality publications ((30), (31), (32), (33), (34), (35,36), (37), (38), (39), (40).

Plants' metabolic processes result in a wide range of organic chemicals. Because they interact with other plants, animals, and bacteria, these secondary metabolites are essential to the plant's own life. Researchers have spent a lot of time studying flavonoids, a class of secondary metabolites found in plants, because they have beneficial qualities like anti-cholinesterase action, anti-inflammatory activity, radical scavenging activity, etc. As phytoalexins, signal molecules, allopathic substances, detoxifying agents, and antimicrobial defense compounds, they also play a significant role. As they disrupt nucleic acids and proteins, these flavonoids are also claimed to have insecticidal and antibacterial properties. They are crucial substances for creating pesticides for the agricultural sector (41).

Additionally, Lipinski's rule of five for flavonoids was examined. Myricetin and vitexin showed promising results despite breaking just one of Lipinski's rules. The examined flavonoids can be regarded as strong candidates in the process of developing drugs to combat hyperglycemia, despite the fact that several parameters were not reached by them (41,42).

Rats fed a cafeteria meal were used as test subjects for the effects of the ethanolic extract of A. speciosa root. The extract demonstrated a reduction in obesity in experimental mice by considerably lowering the serum levels of leptin, total cholesterol, low density lipoprotein, and triglycerides.(28,43).

4. CONCLUSION

The present study shows that streptozotocin alters the glucose metabolism in rats which were treated by A.nervosa.In further studies, molecules or genes related to insulin resistance need to be studied to prove A.nervosa as a potential anti-diabetic agent.

Conflict of Interest:
The authors hereby declare that there is no conflict of interest in this study.

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Author Contribution:
A) Jeshurun Jegan - contributed in designing the study, execution of the project, statistical analysis, manuscript drafting.
B) Dr. Selvaraj - contributed in designing the study, execution of the project, statistical analysis, manuscript drafting.
C) Dr.V.Vishnupriya - contributed in study design, guiding the research work, manuscript correction.
D) Dr. Gayathri R - study design, statistical analysis, manuscript proofreading and correction.
E) Dr. Kavitha S - study design, statistical analysis, manuscript proofreading and correction.

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