Effects of Argyreia Nervosa on IRS/AKT/GLUT4 Signalling Molecules in the Adipose Tissue of STZ Induced Experimental Diabetic Rats

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Abstract

Introduction:

Argyreia nervosa is a perennial climbing vine native to the Indian subcontinent and introduced to numerous areas worldwide. The common name of Argyreia nervosa is elephant creeper. They are well known for their nootropic antioxidant, antiinflammatory and antimicrobial and central nervous depressant activities. A.nervosa has the potency to regulate lipid metabolism in adipose tissue by regulating leptin and adiponectin in diabetic conditions. IRS-I/ AKT/ GLUT-4 signaling regulates the metabolism of adipose tissues by promoting glucose utilization, protein synthesis and lipogenesis.Insulin stimulated the utilization of approximately 10% of glucose in adipose tissue .In this study the effects of A.nervosa on IRS-1/ AKT/GLUT-4 signaling molecules in adipose tissue of STZ induced experimental diabetic rats will be observed.

Materials and Methods:

A.nervosa plants were collected . Roots of the plant were powdered soxhlet extracted with 70 % ethanol.Diabetes was induced in rats by a single intraperitoneal administration of STZ. They were then grouped into 3 groups of six animals each and treated oral administration for 15 days .

Results and Conclusion:

The fasting blood glucose level was more for STZ induced diabetic rats . But the FBG was almost equal for the control group and group III(diabetic rat + oral administration of A.nervosa 500 mg/ kg/ day) rats. The A.nervosa treated group showed decrease in FBG serum insulin when compared to the control group. From the results obtained it can be concluded that A.nervosa plant shows

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potential anti diabetic activity on IRS-1/AKT/GLUT-4 signaling molecule in the adipose tissue of STZ induced experimental diabetic rats.

Keywords: Innovative technique, Argyreia nervosa, IRS, -1/AKT/ GLUT-4, novel method, diabetes.

1. INTRODUCTION

Diabetes mellitus is a metabolic disorder that causes high blood sugar (1). The inadequate secretion or defect in insulin function leads to an improper glucose utilization by cells. The insulin dependent type 1 diabetes is an autoimmune disorder that is characterized by complete loss of functioning pancreatic beta-cell and it requires insulin administration.(2).

The non-insulin-dependent type two diabetes results when the body cannot use the insulin it produces.Several complications were also associated with diabetes. These include nephropathy,neuropathy ,cardiomyopathy ,retinopathy, stroke,coronary heart disease tuberculosis and cancer.(3)Insulin and numerous oral antidiabetic medications such sulfonylureas, biguanides, -glucosidase inhibitors, and glinides are currently available as treatments for diabetes. Because these goods are expensive and difficult to find in impoverished nations like India, plants have been employed as a source of medications for the treatment of diabetes. It has been discovered that many indigenous Indian medicinal plants, which are widely available and have minimal adverse effects, are helpful in the effective control of diabetes.(4).

Since ancient times plants are traditionally used in treatment of numerous health condition disorders and kept playing a pivotal role in modern drug discovery furnishing novel chemical leads with limited side-effects. (3,5)During the last decades resurgence in the use of herbal medicine and products could be considered as a revolutionary shift in classical medicine. Currently many herbal formulations are approved and widely used for their potential antidiabetic activity due to their lower side effects low-cost and higher bioavailability compared to most analogues of synthetic origins.(5).

Argyreia nervosa is a perennial climbing Vine native to the Indian subcontinent. The common name of Argyreia nervosa is elephant creeper. They are well known for their nutropic ,antioxidant, anti-inflammatory, antimicrobial and central nervous depressant activities. Argyreia nervosa has the potency to regulate lipid metabolism in adipose tissue by regulating leptin and adiponectin in diabetic conditions the IRS1/ AKT/GLUT-4 signaling molecules regulate the metabolism of adipose tissues by promoting glucose utilization, protein synthesis and Lipogenesis.(6)Due to the plant's extensive variety of clinical benefits, including its antiviral, antibacterial, anti-fungal, and anti-inflammatory qualities, it has traditionally been used therapeutically. (7)Additionally, it contains spermatogenic, age-maintaining, and rejuvenating properties. The plant's highest concentration of psychotropic substances is found in the seeds.The plant also includes a variety of vitamins and minerals, including potassium, zinc, and vitamin E. Because of its phytoconstituents, which include flavonoids, tannins, saponins, polyphenols, lignins, and quercetin, the testicular cells will be protected from oxidative stress and encouraged to remain viable.(8).

The leaves of A. nervosa have been used to treat swellings and boils. It is utilized externally for eczema, etches, ringworm infections, and skin conditions. Its seeds have hypotensive, spasmolytic, and tonic properties.(9). Our team has extensive knowledge and research experience that has translate into high quality publications (10–19))(20–29)).

Hence in this experimental study we analyzed the effect of Argyreia nervosa on IRS-1/AKT/ GLUT-4 signaling molecules in the adipose tissue of streptomycin induced experimental diabetic rats.

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 (Kokate 2001).

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
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 crossreactivity 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 4°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 by the method of Fourney et al. (1988). 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 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

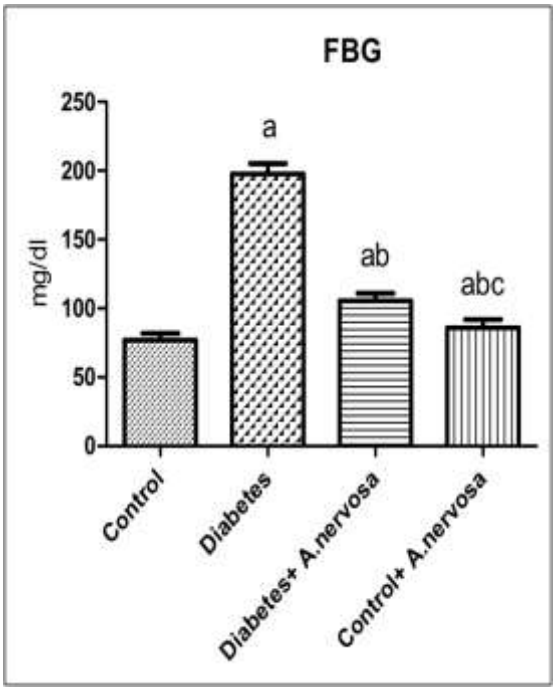

Fig:1 The fig 1 shows that diabetic rats show high fasting blood glucose level, whereas the A.nervosa treated group shows a decrease in FBG compared to control group.

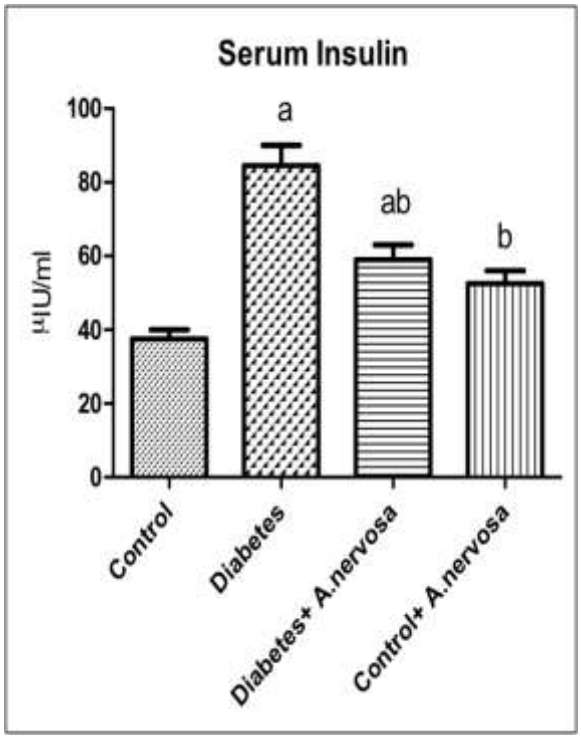

Fig:2 The fig 2 shows that diabetic rats show high serum insulin level, Whereas the A.nervosa treated group shows serum insulin compared to control group.

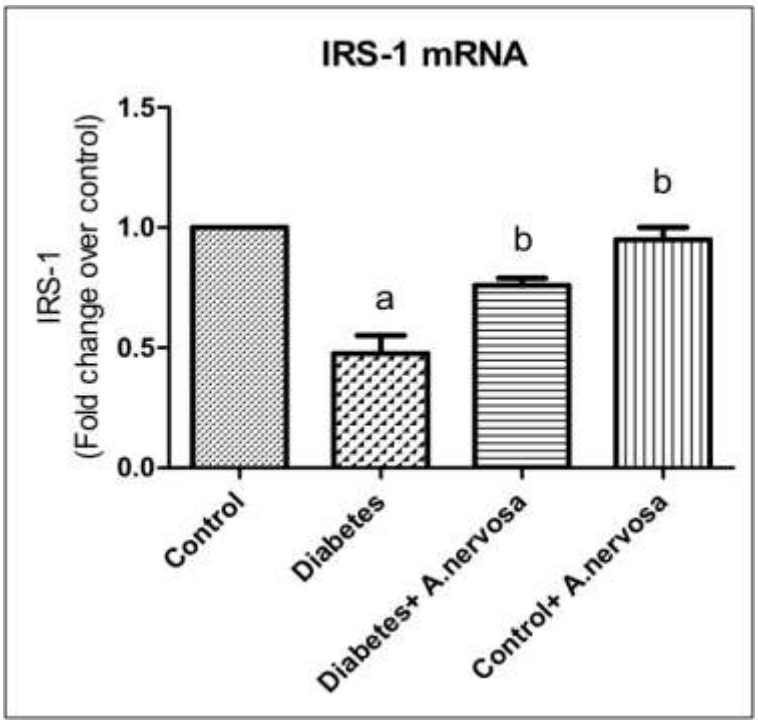
IRS-1 mRNA
IRS-1
(Fold change over control)
1.5
1.0
0.5
0.0
a
b
b
Control
Diabetes
Diabetes+ A.nervosa
Control+ A.nervosa

Fig:3

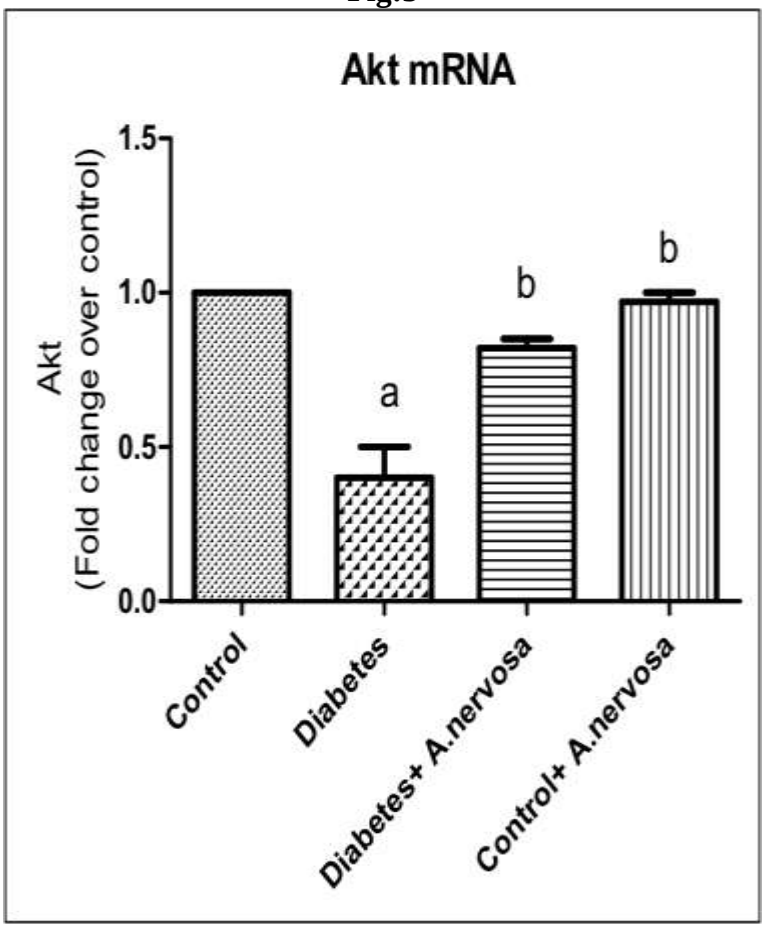
Akt mRNA
Akt
(Fold change over control)
1.5
1.0
0.5
0.0
a
b
b
Control
Diabetes
Diabetes+ A.nervosa
Control+ A.nervosa

Fig:4

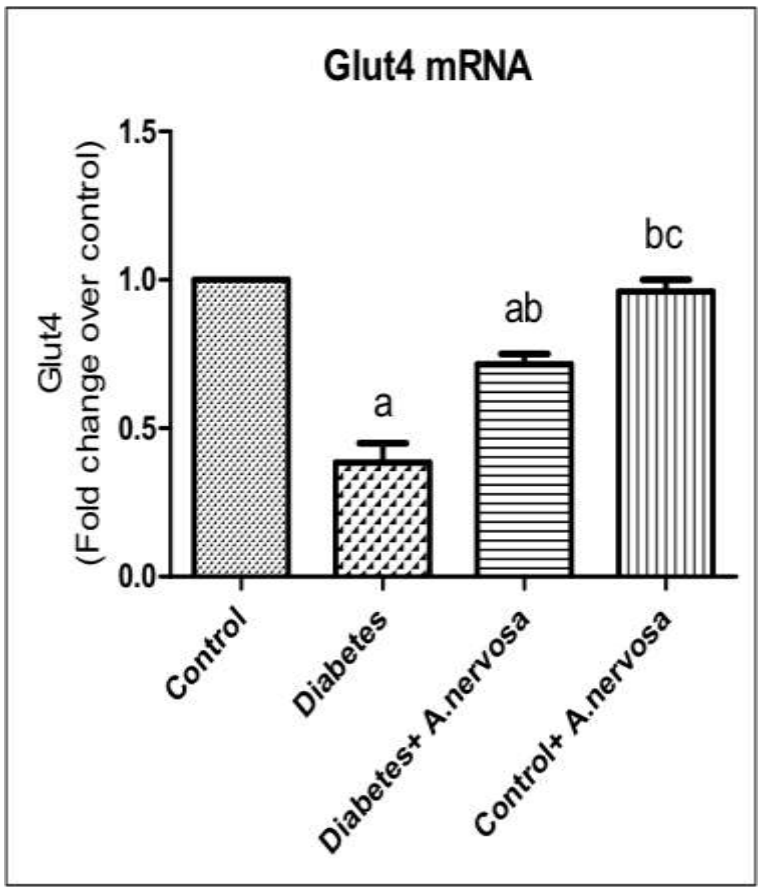

Fig:5

4. DISCUSSION

In the present study the diabetic rats showed a significant increase in fasting blood glucose and serum insulin, whereas the Argyreia nervosa treated group showed the decrease in fasting blood glucose and serum insulin when compared to the control group. The mRNA levels of IRS-1/AKT/GLUT-4 significantly increased in A.nervosa treated group compared to diabetic group.

Argyreia nervosa supplementation had the following favorable benefits in diabetic rats.(a) a considerable reduction in blood glucose, urine glucose and serum fructosamine levels and attenuation of structural arrangements of the pancreatic islets.(b) improvement in insulin secretion and insulin sensitivity and increase of anti-inflammatory cytokine and reduction of pro-inflammatory one.(c) activation of insulin signaling pathway in the adipose tissue .(30).

Since it mimics various metabolic disorders seen in human diabetes the STZ diabetes induced rat model is also known as an ideal model to look for possible therapeutics(31). In STZ induced diabetic rats we expected that insulin levels would be significantly lower and glucose levels would be significantly higher. (32)The Argyreia nervosa decreases the elevated blood glucose and increases the diminished insulin level in diabetic rats without affecting normal glucose level. (32,33)The study showed that Argyriea nervosa has glucose lowering effects and improved the response to injected insulin and genetic model of type two diabetes.(34).

In a few previous studies both normal rats and rats with diabetes induced by alloxan, shows that A. nervosa leaves extract given topically accelerates wound healing more significantly than oral treatment. Topical application of ethanol extract of A. nervosa leaves plays a major role in wound healing in normal and diabetic animals. It was also found that the ethanolic extract of A. nervosa is more effective topically compared to oral preparation.This is true even in cases where wound healing would otherwise be delayed.(34)Traditional medicine, medicinal herbs, and natural plants are increasingly being used because they are more effective therapeutically, easily accessible, socially acceptable, and have less or no concurrent side effects than modern medicine.(5).

In another study In vitro antiinflammatory activity was carried out by inhibiting the heat induced albumin denaturation,membrane stabilization and protein denaturation activity. The samples were studied for their effect on inhibition of glycosylation of hemoglobin ,glucose transport across yeast cells and α- Amylase inhibition. From the results of the study, it is inferred that A.nervosa root possesses good anti-inflammatory and anti-diabetic activity.(35). Our team has extensive knowledge and research experience that has translate into high quality publications (36), (37), (38), (39), (40,41), (42), (43), (44), (45), (46), (47), (48), (49).

From our study we found that the diabetic rats showed a significant increase in fasting blood glucose and serum insulin, whereas the Argyreia nervosa treated group showed the decrease in fasting blood glucose and serum insulin when compared to the control group. The mRNA levels of IRS-1/AKT/GLUT-4 significantly increased in A.nervosa treated group compared to diabetic group.

5. CONCLUSION

From the results obtained it can be concluded that Argyreia nervous plant shows potential antidiabetic activity on IRS-1/AKT/GLUT-4 signaling molecules in the adipose tissue of STZ induced experimental diabetic rats. Furthermore research based on insulin signaling molecules and gene expression analysis is needed to prove the potential antidiabetic activity of Algeria nervosa.

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

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Author Contribution:
A) Karunya Arumugam - 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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