Journal of Namibian Studies, 33 (2023): 5942-5950 ISSN: 2197-5523 (online)

Effect Of Argyreia Nervosa On The Expression Of Growth Factor Signaling In The Skeletal Muscle Of Streptozotocin-Induced Experimental Diabetic Rats

Ojastha B L[1], Selvaraj J[2], Kavitha S[3*], Veeraraghavan Vishnu Priya[4], Gayathri R[5]

Abstract:

Introduction: Diabetes mellitus, is a chronic disorder marked by a lack of insulin, where there is a role of the metabolic and hemodynamic pathway leading to an increase or relative deficit of growth factors in target tissues. Argyreia nervosa has α-amylase inhibitory flavonoids (viexin, rutin, myricetin & isoquercetin), which exert their anti-oxidant and anti-inflammatory activities by maintaining antioxidant levels. This study aimed to assess the antihyperglycemic activity and effect on growth factor signalling proteins IGF and TGF of Argyreia nervosa. Methods: Rats that showed hyperglycaemia (blood glucose level >250 mg/dl) were selected for the experimental study. Animals were grouped into 4 groups of six animals each as Normal rats, Diabetic rats, Diabetic rats with oral administration of plant root extract and Normal rats with oral administration of root extract and the experimental period was 5 days. At the end of the experimental period, the rats were

[1]Department of Biochemistry, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences (SIMATS), Saveetha University, Chennai - 600077, Tamil Nadu, India
Email ID: 152101015.sdc@saveetha.com

[2] Department of Biochemistry, Saveetha Dental college and Hospitals, Saveetha Institute of Medical and Technical Sciences, (SIMATS), Saveetha University, Chennai - 600077, Tamil Nadu, India
Email ID: selvarajj.sdc@saveetha.com

[3*]Department of Biochemistry, Saveetha Dental college and Hospitals, Saveetha Institute of Medical and Technical Sciences, (SIMATS), Saveetha University, Chennai - 600077, Tamil Nadu, India
Email ID:kavithas.sdc@saveeetha.com

[4] Department of Biochemistry, Saveetha Dental college and Hospitals Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai - 600077, Tamil Nadu, India
Email ID: vishnupriya@saveetha.com

[5] Department of Biochemistry, Saveetha Dental college and Hospitals Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai - 600077, Tamil Nadu, India
Email ID: gayathri.sdc@saveetha.com

euthanized and gene expression analysis was done for IGF and TGF. Results: The expression of growth factors increased after induction of STZ thus enhancing oxidative stress and thus implicated in diabetes conditions. Diabetes enhances oxidative stress and induces the expression of TGF & IGF. Conclusion: Growth factor expression is used as a marker of diabetes and reversal of growth factor expression was observed after the application of plant root extract, which confirms it as a therapeutic target for diabetes.

Keywords: Plant extract, fasting blood glucose, diabetic mellitus, gene expression

INTRODUCTION:

Diabetes, as a metabolic disorder is a condition that disrupts cellular function, or as a pathological entity causing specific tissue and organ functional and structural damage. It is marked by a lack of insulin and it changes the growth factor levels in cell (1) (2). Growth factors have a major role in integrating tissue physiology, embryology, growth maturation, and tissue repair. In tissues affected by diabetes, growth factors are induced by a relative deficit or excess. These factors are endogenously produced polypeptides that affect cellular functions, most commonly by inducing cell hyperplasia or tissue hypertrophy resulting in the growth of the organism, imbalances of their expression leading to derangements of cellular metabolism & proliferation. In diabetes, there is a role of the metabolic and hemodynamic pathways leading to an increase in growth factors in the target tissues (3). Diabetic related growth factors include Fibroblast growth factor 21 (FGF 21) which regulates glucose & lipid metabolism, Transforming Growth Factor B1 whose increase or decrease is associated with diabetic nephropathy & retinopathy, Insulin-like Growth Factor 1 (IGF 1) is a natural occurring single chain polypeptide associated with diabetes (4,5).

Streptozotocin (STZ) is a glucosamine nitrosourea compound derived from Streptomyces chromogens, damaging β-cells resulting in hyperinsulinemia and hyperglycemia. It enters the B cells via a glucose transporter (GLUT 2) and causes alkylation of DNA, DNA damages induced activation of poly ADP ribosylation, this leads to depletion of cellular NAD + & ATP. Enhanced ATP dephosphorylation after Streptozotocin treatment supplies a substrate for xanthine oxidase, resulting in the formation of hydrogen peroxide and hydroxyl radicals (dismutation of radicals) (fentan reaction). The action of ROS with a massive increase in cytosolic calcium concentration causes rapid destruction of B cells (necrosis) (6).

Argyreia nervosa is a woody climbing shrub found throughout India. Roots of *A. Nervosa* are used traditionally as aphrodisiac, rejuvenating, intellect promoting, and bronchitis. Phytochemical investigations on

the roots of this plant have resulted in the isolation of a range of biologically active substances like Flavonoid sulphates 4 Stigmasteryl p-hydroxycinnamate and Coumarin 5 and many phenolic compounds (7). A study reported the isolation, characterization, and antimicrobial antituberculosis efficacy of flavonoid sulfates and other fractions of *A. speciosa* (8).

Our team has extensive knowledge and research experience that has translated into high-quality publications. The objective of the present study is to determine the effect of selected plant root part ethanolic extract in the expression of growth factor signaling molecules in the skeletal muscle of STZ-induced diabetic rats.

MATERIALS & METHODS:

Chemical

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). The total RNA isolation reagent (TRIR) was purchased from Invitrogen, USA. The reverse-transcriptase enzyme (MMuLv) was purchased from New England Biolabs (NEB), USA, and the GoTaq Green master mix was purchased from Promega, USA. IGF and TGFβ and β-actin primers were purchased from Eurofins Genomics India Pvt Ltd, Bangalore, India.

Plant collection and extract preparation

The species will be verified at Anna Siddha Hospital in Chennai, Tamil Nadu, using plant root powder obtained from a pharmacy. Roots part used for this study. The fine powder was mixed with 70% ethanol, The extract was then filtered with Whatman no. 1 filter paper. Then a rotary vacuum evaporator was used for the preparation of the concentrated extract. 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 with 180–200 g of weight) 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.

Diabetic induction

Diabetes was induced in rats by a single intraperitoneal (ip) administration of STZ (55 mg/kg) dissolved in 0.1 M citrate buffer, pH 4.5. 48 hours later, blood collected from the rat tail tip and then using of digital glucometer blood glucose level will be measured. The rats that showed hyperglycemia (blood glucose level >250 mg/dl) were selected for experimental study (Shiv 2010).

Grouping

Animals were grouped into 4 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 ethanolic root extract 500 mg/kg/day, Group IV - normal rat + oral administration of ethanolic root extract 500 mg/kg/day

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.

Total RNA isolation, cDNA conversion and real-time

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 4ɵ 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 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.

S.no	Gene name	Sequences
1.	IGF	TGTCGGGATATCTTTCCGGC GCTTTTACTTCAACAAGCCCACA
2.	TGFβ	AAATGGGCTCCCTCTCATCAGTT TCTGCTTGGTGGTTTGCTACGAC
3.	β-actin	AAGTCCCTCACCCTCCCAAAAG AAGCAATGCTGTCACCTTCCC

Table 1. Primer sequence details

Statistical analysis

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

RESULTS:

Effect of ethanolic root extract on FBG and Serum insulin in stz-induced diabetic rats

Fasting blood glucose are shown in figure 1. Rats administered root extract significantly (*p<0.05*) increased blood glucose levels in rats, compared to the control.

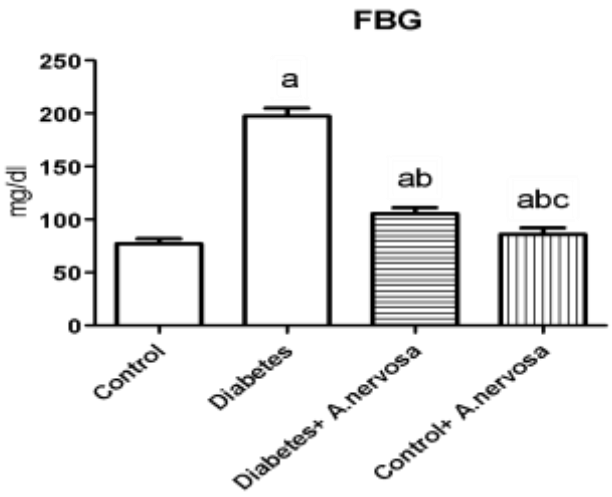

Figure 1: FBG levels of normal and treated groups. Each bar represents the mean ± S.E.M of 6 animals. a-compared to control; b-compared to diabetes group. c-compared with extract treated group. Significance was considered at the levels of *p<0.05.*

Effect of ethanolic root extract on mRNA expressions in STZ-induced diabetic rats

mRNA expressions of IGF and TGF-β

Figures 2 and 3 depict the effects of oral administration of ethanolic extracts on IGF and TGFβ mRNA expressions. We found that mRNA expression of IGF and TGFβ gradually increased in the treatment groups when compared to the control.

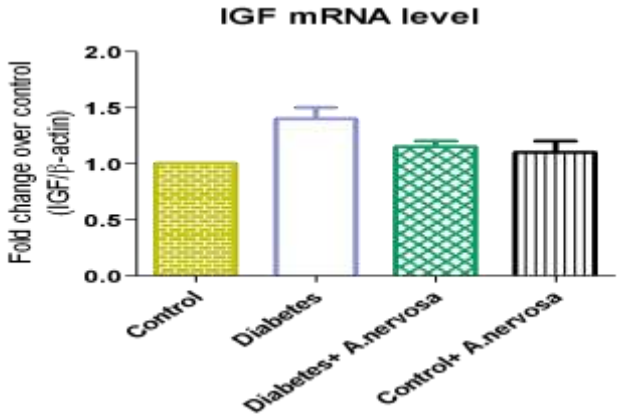

Figure 2 : IGF mRNA levels of normal and treated groups. Each bar represents the mean ± S.E.M of 6 animals. a-compared to control; b-compared to diabetes group. c-compared with extract treated group. Significance was considered at the levels of *p<0.05*.

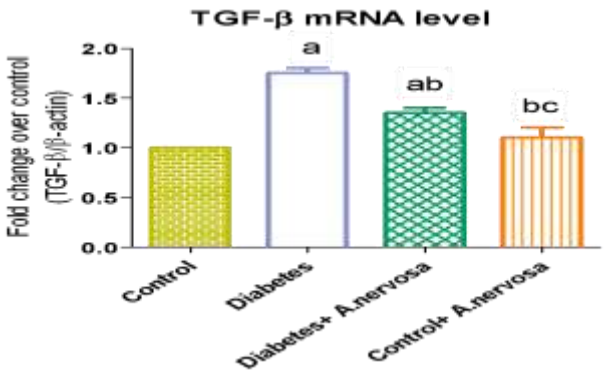

Figure 3: TGFβ mRNA levels of normal and treated groups. Each bar represents the mean ± S.E.M of 6 animals. a-compared to control; b-compared to diabetes group. c-compared with extract treated group. Significance was considered at the levels of $p<0.05$.

DISCUSSION:

Herbs are utilized in medication because of their minimal or no negative impacts (9). The bioactives abundant in herbs may be responsible for their health-promoting qualities. Several studies have found that the presence of various bioactives in medicinal herbs contributes to their health-promoting qualities (10) (11). For centuries, many plants have been considered a fundamental source of potent antidiabetic drugs. In developing countries, particularly, medicinal plants are used to treat diabetes to overcome the burden of the cost of conventional medicines to the population (12).

Nowadays, treatments of diseases including diabetes using medicinal plants are recommended, because these plants contain various phytoconstituents such as flavonoids, terpenoids, saponins, carotenoids, alkaloids, and glycosides, which may possess antidiabetic activities (13,14). Also marked by the combined action of biologically active compounds (i.e., polyphenols, carotenoids, lignans, coumarins, glucosinolates, etc.) leads to the potential beneficial properties of each plant matrix, and this can represent the first step for understanding their biological actions and beneficial activities (15). Generally, the main current approaches of study of the interactions of phytochemicals can be classified: (i) model system development of interactions (ii) study of extractable and non extractable compounds; or (iii) characterization of biologically active compound-rich extracts. Most of these plants contain bioactive compounds such glycosides, alkaloids, terpenoids, flavonoids, carotenoids, etc., that are frequently implicated as having an antidiabetic effect (8).

Salma et al did a study on *Cassia auriculata* root against high-fat diet in STZ-induced type-2 diabetes in C57BL/6 Mice. In that study they concluded that treatment of the *Cassia auriculata* has the ability to

alter the FBG, body weight and histopathology modification in tissue level (16). Similarly in our study also we got results like alteration in blood glucose and tissue levels of mRNA expression. The present data suggested that FBG levels were significantly alter in the treatment groups when compared to the control, and mRNA expressions of TGF & IGF growth factor levels significantly reduced hyperglycemia in multiple dose. Thus this study which confirms the therapeutic effect of A.nervosa in diabetic rats

CONCLUSION:

The present study concludes the beneficial effect of *Argyreia nervosa* roots in the control of blood glucose level in normal and diabetic rats. The study confirms the rational basis for its use in traditional medicine for the treatment of diabetes. Further phytochemical and pharmacological investigations would characterize active phytoconstituents and establish the exact mechanism of their hypoglycemic action. More research is required in the future to understand the impact in the plant based drug on animal model and cell line study.

REFERENCES :

1. Bhaskar A, Vidhya VG, Ramya M. Hypoglycemic effect of Mucuna pruriens seed extract on normal and streptozotocin-diabetic rats [Internet]. Vol. 79, Fitoterapia. 2008. p. 539–43. Available from: http://dx.doi.org/10.1016/j.fitote.2008.05.008
2. Vishaka S, Sridevi G, Selvaraj J. An in vitro analysis on the antioxidant and anti-diabetic properties of Kaempferia galanga rhizome using different solvent systems. J Adv Pharm Technol Res. 2022 Dec;13(Suppl 2):S505–9.
3. Sneka S, Sinduja P, Priyadharshini. A COMPARATIVE STUDY ON SALIVARY pH IN DIABETIC PATIENTS WITH PERIODONTITIS AND WITHOUT PERIODONTITIS. Journal of Pharmaceutical Negative Results. 13(3):1490–9.
4. Prasad M, Jayaraman S, Rajagopal P, Veeraraghavan VP, Kumar PK, Piramanayagam S, et al. Diosgenin inhibits ER stress-induced inflammation in aorta via iRhom2/TACE mediated signaling in experimental diabetic rats: An in vivo and in silico approach [Internet]. Vol. 358, Chemico-Biological Interactions. 2022. p. 109885. Available from: http://dx.doi.org/10.1016/j.cbi.2022.109885
5. Association Between Right Upper Molar Involvement And Diabetes Mellitus In Subjects With Chronic Periodon-titis [Internet]. [cited 2023 Sep 4]. Available from: https://www.academia.edu/download/73119179/IJDOS_2377_8075_08_6061.pdf
6. Sarfraz M. Diabetes Mellitus and Protective Approaches of Medicinal Plants: Present Status and Future Prospects [Internet]. Phytotherapy in

the Management of Diabetes and Hypertension: Volume 3. 2020. p. 38–60. Available from: http://dx.doi.org/10.2174/9789811459139120030004

7. Pullaiah T, Chandrasekhar Naidu K. Antidiabetic Plants in India and Herbal Based Antidiabetic Research. Daya Books; 2003. 350 p.
8. Subramanyam GK, Gaddam SA, Kotakadi VS, Palithya S, Penchalaneni J, Challagundla VN. leaf extract mediated silver nanoparticles and evaluation of their antioxidant, antibacterial activity, anticancer and apoptotic studies in KB oral cancer cell lines. Artif Cells Nanomed Biotechnol. 2021 Dec;49(1):635–50.
9. Karthik EVG, Priya V. V, Gayathri. R, Dhanraj Ganapathy. Health Benefits Of Annona Muricata-A Review. Int J Dentistry Oral Sci. 2021;8(7):2965–7.
10. Johnson J, Shanmugam R, Lakshmi T. A review on plant-mediated selenium nanoparticles and its applications. J Popul Ther Clin Pharmacol. 2022 Jan 6;28(2):e29–40.
11. Craig WJ. Health-promoting properties of common herbs. Am J Clin Nutr. 1999 Sep;70(3 Suppl):491S – 499S.
12. Eddouks M. Phytotherapy in the Management of Diabetes and Hypertension: Volume 3. Bentham Science Publishers; 2020. 234 p.
13. Habbu PV, Mahadevan KM, Kulkarni VH, Marietta P, Pratap V, Thippeswamy BS, et al. Antidiabetic activity of Argyreia speciosa (sweet) (Burm.f.)Boj. in normoglycemic and Streptozotocin-induced diabetic rats [Internet]. Vol. 10, Oriental Pharmacy and Experimental Medicine. 2010. p. 90–102. Available from: http://dx.doi.org/10.3742/opem.2010.10.2.090
14. Habbu PV, Mahadevan KM, Shastry RA, Manjunatha H. Antimicrobial activity of flavanoid sulphates and other fractions of Argyreia speciosa (Burm.f) Boj. Indian J Exp Biol. 2009 Feb;47(2):121–8.
15. Durazzo A, Lucarini M, Heinrich M. Editorial: Dietary Supplements, Botanicals and Herbs at the Interface of Food and Medicine. Front Pharmacol. 2022 Jul 4;13:899499.
16. Salma B, Janhavi P, Muthaiah S, Veeresh P, Santhepete Nanjundaiah M, Divyashree S, et al. Ameliorative Efficacy of the Root Against High-Fat-Diet + STZ-Induced Type-2 Diabetes in C57BL/6 Mice. ACS Omega. 2021 Jan 12;6(1):492–504.