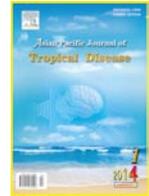




Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Disease

journal homepage: www.elsevier.com/locate/apjtd

Document heading

doi: 10.1016/S2222-1808(14)60429-1

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Evaluation of antidiabetic property of *Andrographis paniculata* powder in high fat and sucrose-induced type-2 diabetic adult male rat

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Scientific validation of medicinal plants would help to popularize the same among the public. The present study has a clear approach in studying the mechanism of action in the antidiabetic effect of the plant, validating its antidiabetic property. The results are interesting and suggest that the antidiabetic activity of *A. paniculata* may be attributed to its antioxidant, antilipidemic and regulation of insulin signal transduction.

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ABSTRACT

Objective: To evaluate the antidiabetic effect of the aerial part of *Andrographis paniculata* (*A. paniculata*) powder (500 mg/kg body weight) in high fat and sucrose-induced type-2 diabetic rat model.

Methods: The fasting blood glucose, oral glucose tolerance test, serum insulin, lipid profile, mRNA and protein levels of insulin signaling molecules, 14C-2 deoxy glucose uptake and 14C glucose oxidation in liver were checked.

Results: In the type-2 diabetes-induced group, the fasting blood glucose, oral glucose tolerance, serum insulin, lipid profile, glucose uptake and oxidation, Akt and glucose transporter 2 mRNA, insulin receptor and glucose transporter 2 protein (both cytosolic and plasma membrane) and phosphorylation of insulin receptor substrate 1 and Akt were impaired. *A. paniculata* was able to successfully reinstate this impairment. In addition to this, *A. paniculata* did not cause a hypoglycemic condition in normal rat, affirming its activity in hyperglycemic state alone.

Conclusions: *A. paniculata* possesses significant antidiabetic and antihyperlipidemic activities in high fat and sucrose-induced type-2 diabetic rat and the molecular actions at the level of insulin signaling molecules in liver reinforce it.

KEYWORDS

Diabetes mellitus, Insulin resistance, Antidiabetic, Medicinal plant, *Andrographis paniculata*, Antihyperlipidemic, Insulin signaling, High fat diet, Sucrose, Liver

1. Introduction

Type-2 diabetes mellitus (T2DM) is characterized by fasting and postprandial hyperglycemia and relative insulin insufficiency[1]. T2DM is associated with substantial morbidity and mortality[2]. Unlimited supply of energy-dense food and technologies that encourage sedentary behavior have introduced a new threat to the survival of our species: obesity and its co-morbidities. The foremost co-morbidities is T2DM, which is projected to afflict 400 million people worldwide by 2030[3]. The treatment goals for T2DM patients are effective

control of blood glucose, blood pressure, and lipids[4]. At present, therapy for T2DM relies mainly on several approaches intended to reduce the hyperglycaemia itself, including sulphonylureas (and related insulin secretagogues), which increase insulin release from pancreatic islets; metformin, which acts to reduce hepatic glucose production; peroxisome proliferator-activated receptor- γ agonists (thiazolidinediones), which enhance insulin action; α -glucosidase inhibitors, which interfere with gut glucose absorption; and insulin itself, which suppresses glucose production and augments glucose utilization. These therapies have limited efficacy, limited

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Foundation Project: Supported by the University of Madras, Chennai, India (Grant No. URF/Fellowship/2009/1042, dated 10/12/09)

Article history:

Received 26 Nov 2013

Received in revised form 30 Nov, 2nd revised form 5 Dec, 3rd revised form 8 Dec 2013

Accepted 24 Jan 2014

Available online 28 Jan 2014

tolerability and significant mechanism-based side effects[5].

Despite the impressive advances in health sciences and medical care, there are many patients who are using alternative therapies alone or complementary to the prescribed medication[6]. Plants are being rediscovered because of growing awareness of unwanted side effects and other aspects of allopathic medicines[7]. There is a growing interest in herbal remedies because of their effectiveness, minimal side effects in clinical experiences and relatively low costs. Herbal drugs or their extracts are prescribed widely; even when their biological active compounds are unknown as they are being traditionally used[8]. Several species of medicinal plants are used in the treatment of diabetes mellitus[9]. Few antidiabetic herbs are *Momordica charantia*, *Cephalandra indica*, *Syzygium jumbolanum*, *Gymnema sylvestre* and *Andrographis paniculata* (*A. paniculata*).

A. paniculata is one of the most commonly used plants in the traditional systems of Unani, Siddha and Ayurvedic medicines. It is called Creat in English and is known as the king of bitters. Presence of active ingredients from diterpenoids group such as andrographolide, neoandrographolide and 14-deoxy-11,12-didehydroandrographolide has given incredible unique medicinal properties to the plant[10]. *A. paniculata* has been reported to have numerous medicinal properties[11]. Moreover, in Compendium of Medicinal and Aromatic Plants, *A. paniculata* is reported as a leading medicinal plant in treating diabetes, high blood pressure and various other ailments, traditionally[12]. Although *A. paniculata* has been used as antidiabetic drug in traditional systems of medicine, its mode of action has not been evaluated. On this account, the dry powder of *A. paniculata* (aerial part) was chosen for the present study to conventionalize its antidiabetic property in type-2 diabetic adult male Wistar rat.

2. Materials and methods

2.1. Chemicals and reagents

All chemicals and reagents used in the present study were of analytical grade (analytical reagents) and they were purchased from Amersham Biosciences Ltd. (UK) and Sisco Research Laboratories, Mumbai. ^{14}C -2-deoxy glucose and ^{14}C glucose were purchased from Board of Radiation and Isotope Technology, Mumbai. The glucose kit was purchased from CPC Diagnostics, Spain.

2.2. Animals

The present experimental study was approved by the Institutional Animal Ethics Committee (IAEC No: 01/022/2010; dated. 30/03/2010). Adult male Wistar albino rats (90–120 days old weighing 120–150 g) were obtained and maintained in clean polypropylene cages at the Central Animal House Facility, University of Madras (Taramani campus).

2.3. Induction of type-2 diabetes

Rats were subjected to 30 d of high-fat diet containing cholesterol 1.5%, cholic acid 0.5%, coconut oil 30%, standard rat feed 68%, and 30% sucrose through drinking water. On the 28th day of treatment, after overnight fasting, blood glucose was checked and the rats which had blood glucose above 120 mg/dL were chosen as type-2 diabetic rats.

2.4. Plant material

The plant was collected from the local area, Chennai, Tamil Nadu. It was authenticated by Dr. T. Aravind, Assistant professor, Department of Medicinal Botany, National Institute of Siddha, Chennai-600047. A voucher specimen has been deposited at the museum of the department. The aerial part of *A. paniculata* was shade dried, finely ground, dissolved in water and used for treatment.

2.5. Formulation and plant administration

The plant powder was dissolved in water just before every administration [500 mg/(kg · d)] through oral intubation.

2.6. Experimental design

The following experimental design was framed and accordingly the rats were subjected to treatment for a period of two months. Healthy adult male Wistar rats were divided into the following groups: Group I: control (vehicle treated); Group II: rats were made diabetic (type-2) after 4 weeks of high fat diet feed and sucrose through drinking water (30%); Group III: diabetic (type-2) rats treated with *A. paniculata* 500 mg/(kg · d) (orally) for 30 d; Group IV: control rats treated with *A. paniculata* 500 mg/(kg · d) (orally) for 30 d; Group V: diabetic (type-2) rats treated with metformin [50 mg/(kg · d)] orally for 30 d.

Two days prior to killing, control and experimental animals were subjected to oral glucose tolerance (OGT) test after overnight fasting. At the end of 30 d, blood was collected and animals were perfused with normal saline under ether anesthesia. Liver were dissected out for the assessment of various parameters.

2.7. Fasting blood glucose (FBG)

Blood glucose was estimated using On-Call Plus blood glucose test strips (ACON Laboratories, Inc. San Diego, USA Life Scan Inc. Milpitas, USA) after overnight fasting. Blood glucose was assessed by nicking the tip of the rat tail and results are expressed as mg/dL.

2.8. OGT test

Blood glucose was estimated using On-Call Plus blood glucose test strips at various time periods (60, 120 and 180 min) after giving the oral glucose load (10 mL/kg; 50% w/v). Blood

glucose value before giving glucose is considered as 0 minute value. Results are expressed as mg/dL.

2.9. Radioimmunoassay of insulin

Serum insulin was assayed using 125I-labeled radioimmunoassay kit obtained from DiaSorin (Saluggia, Italy). The limit of detection is 3 μ IU/mL. The percentage cross-reactivity of insulin antibody to rat insulin was 100% and to C-peptide was <0.01%. Intra-assay coefficient of variation was <10.6% and inter-assay coefficient of variation was <10.8%. Results are expressed as μ IU/mL.

2.10. Serum lipid profile and liver and kidney marker enzymes

Serum cholesterol, triglycerides, low density lipoprotein (LDL), high density lipoprotein (HDL), alkaline phosphatase (ALP), aspartic transaminase (AST), alanine aminotransferase (ALT), total bilirubin, urea, creatinine, lactate dehydrogenase (LDH) and γ -glutamate transpeptidase were assessed using assay kits purchased from Spinreact, Spain.

2.11. Gene expression analysis

2.11.1. Isolation of total RNA

Total RNA was isolated from control and experimental samples using total RNA isolation reagent kit obtained from Invitrogen. RNA pellets were mixed with 50 μ L of autoclaved double distilled demineralised water (Milli-Q). The concentration and purity of RNA were determined spectrophotometrically at $A_{260/280}$ nm. The purity of RNA obtained was 1.8. The yield of RNA is expressed in μ g.

RNA was quantified and subjected to cDNA conversion. cDNA was made from mRNA templates using oligodT, dNTPs and reverse transcriptase enzyme. The components were combined in a reverse transcriptase buffer for an hour at 37 °C. The reaction was set as follows: 42 °C for 60 min, 90 °C for 10 min and finally maintained at 4 °C for 5 min. After the reaction, samples were proceeded to the real time polymerase chain reaction (PCR) analysis.

2.11.2. Real time PCR

The reaction mixture was prepared by adding all the components (2x reaction buffer, forward and reverse primers and water) together (45 μ L) except the cDNA templates of the target gene and house-keeping gene, β -actin (the selection of primer sequences was based on previous publications). It is mixed thoroughly and spun down. About 5 μ L of template cDNA for samples, 5 μ L of control DNA for positive control and 5 μ L of water for negative control were taken in separate PCR vials. Then 45 μ L of reaction mixture was added into the reaction vial and the reaction was set up for 35 cycles (95 °C for 5 min, 95 °C for 5 seconds, 60 °C for 20 seconds and 72 °C for 40 seconds).

The results obtained were plotted on a graph by the PCR machine and an amplification curve and melt curve analysis was generated from which relative quantification was calculated.

2.12. Western blot analysis

Plasma membrane and cytosolic fractions from liver were prepared as described previously^[13,14]. Protein concentration was estimated using bovine serum albumin as a standard^[15]. Western blot was done to quantify the protein levels of insulin receptor (IR), insulin receptor substrate (IRS-1), pIRS-1^(Tyr632), Akt, pAkt^(Ser473), glucose transporter 2 (GLUT2) (cytosolic and plasma membrane). Results were normalized with β -actin (the phosphorylated forms were normalized with their respective total proteins).

2.13. Glucose uptake and oxidation

Glucose uptake in liver was estimated by the standard method using ¹⁴C-2-deoxy glucose. Briefly, 10 mg of tissue was incubated in 5 mL of DMEM with 5 mmol/L glucose for 30 min under the condition of 95% air, 5% CO₂. After this, the tissue was washed and incubated for 30 min in glucose free medium for 30 min. Glucose uptake was initiated by the addition of 2 μ L of 0.05 μ Ci ¹⁴C-2-deoxyglucose. At the end of 10 min of incubation, tissue was removed, rapidly rinsed in isotope free medium, homogenized in 2 mL of 5% trichloroacetic acid and placed in the scintillation vials containing 5 mL of scintillation fluid and counted in a beta counter. Results are expressed as count per minute of ¹⁴C-2-deoxyglucose/100 mg tissue.

¹⁴C-glucose oxidation in liver was estimated by the method of Johnson and Turner^[16] and Kraft and Johnson^[17]. Results are expressed as count per minute of ¹⁴CO₂ released/100 mg tissue.

2.14. Statistical analysis

The data were subjected to statistical analysis using One-way analysis of variance (ANOVA) and Duncan's multiple comparison test to assess the significance of individual variations between the control and treatment groups using a computer based software (SPSS 7.5 using Windows student version). The significance was considered at the level of $P < 0.05$.

3. Results

Fasting blood glucose (Figure 1a) was elevated significantly in type-2 diabetes group when compared to control animals. *A. paniculata* treated group significantly reduced the fasting blood glucose level more efficiently than metformin (the standard drug) treated group did. In control+Ap group, *A. paniculata* did not alter when compared to control. In OGT test (Figure 1e) of control rats, glucose concentration rose sharply after glucose load and attained its maximum at 60 min and thereafter it started declining and the level was brought back to normal range by 180 min. In the diabetic group the rise at 60 min was not lowered at 120 min and did not return to its fasting range in 180 min. Also, in the diabetic group, the blood glucose at basal as well as other time points during OGT test showed a significant increase compared to control. The rise in blood glucose level at 60 min in *A. paniculata* administered group was gradually

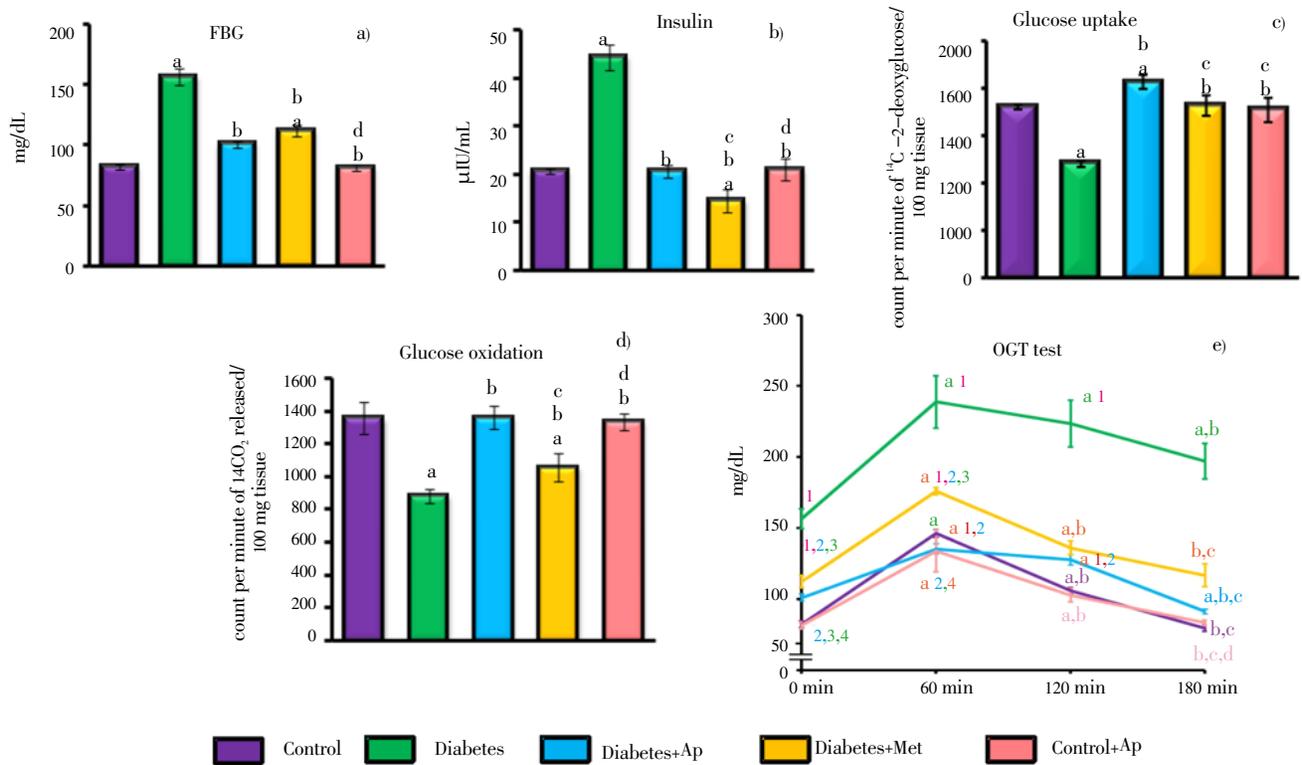


Figure 1. Effect of *A. paniculata* on FBG, insulin, OGT, glucose uptake and oxidation in liver of type-2 diabetic adult male rat. Each bar/point represents Mean±SEM of 6 animals. Significance at $P < 0.05$, a/1– compared with control; b/2– compared with diabetes+*A. paniculata*; c/3– compared with diabetes+metformin; d/4– compared with diabetes+metformin.

reduced at 120 min and reaches its fasting blood glucose range at 180 min. Control+Ap group followed the trend of control groups at all time points.

Serum insulin level (Figure 1b) was increased markedly in the diabetic rats when compared to control, but *A. paniculata* was able to significantly pull down this increase competently with the conventional drug.

A significant decrease of ^{14}C -2-deoxy glucose uptake (Figure 1c) was seen in the diabetic group of both the tissues. *A. paniculata* notably increased it more effectively than metformin when compared to the diabetic group. ^{14}C glucose oxidation (Figure 1d) was significantly decreased in the diabetic group of both the tissues. *A. paniculata* significantly increased it when compared to the diabetic group.

High cholesterol, high triglyceride (TG), high LDL cholesterol and low HDL cholesterol was observed in the diabetic animals of the present study (Table 1). *A. paniculata* ably prevents the dyslipidemia compared to that of metformin. In control+*A. paniculata* group, no change was observed.

Table 1
Effect of *A. paniculata* on serum lipid profile of T2DM rat.

Groups	Cholesterol*	TG*	LDL*	HDL*
Control	49±1	21.0±0.7	11.0±0.8	40±1
Diabetes	95±3 ^a	125±5 ^a	40±3 ^a	24±2 ^a
Diabetes+Ap	80±4 ^{a,b}	90±2 ^{a,b}	14±1 ^b	35±2 ^b
Diabetes+Met	90±1 ^{a,b}	114±4 ^{a,b}	18±1 ^b	34±2 ^b
Control+Ap	50±2 ^{b,c,d}	24±1 ^{b,c,d}	12±2 ^b	39±2 ^b

Each group represents Mean±SEM of 6 animals. Significance at $P < 0.05$. a– compared with control; b– compared with diabetic control; c– compared with diabetes+Ap; d– compared with diabetes+Met; *Units: mg/dL.

In the diabetic group there was increased serum liver (AST, ALT, GGT, ALP, LDH and bilirubin) (Table.2) and kidney (urea and creatinine) (Table 3) function marker enzymes which are indicative of liver and kidney damage. *A. paniculata* was able to significantly lower the serum liver and kidney function markers when compared to the diabetic group.

Table 2
Effect of *A. paniculata* on serum liver function markers of T2DM rat.

Groups	ALP*	ALT*	AST*	Bilirubin [#]	LDH*	GGT*
Control	246±13	13±0.5	9±1	1.4±0.1	128±4	5±0.4
Diabetes	449±7 ^a	46±3 ^a	28±0.8 ^a	6.7±0.4 ^a	578±11 ^a	14±0.6 ^a
Diabetes+Ap	336±5 ^{a,b}	29±0.5 ^{a,b}	14±0.2 ^{a,b}	4.1±0.4 ^{a,b}	293±5 ^{a,b}	7±0.3 ^b
Diabetes+Met	379±9 ^{a,b}	39±2 ^{a,b}	17±1 ^{a,b}	5.1±0.4 ^{a,b}	447±24 ^{a,b,c}	12±0.5 ^{a,b,c}
Control+Ap	270±12 ^{b,c,d}	17±0.8 ^{b,c,d}	10±0.4 ^{b,c,d}	1.5±0.2 ^{b,c,d}	166±8 ^{b,c,d}	6±0.7 ^{b,d}

Each group represents Mean±SEM of 6 animals. Significance at $P < 0.05$, a– compared with control; b– compared with diabetic control; c– compared with diabetes+Ap; d– compared with diabetes+metformin; *Units: U/L; [#]Units: mg/dL.

Table 3
Effect of *A. paniculata* on serum kidney function markers of T2DM rat.

Groups	Urea*	Creatinine*
Control	35±2	0.63±0.05
Diabetes	138±4 ^a	1.57±0.20 ^a
Diabetes+Ap	53±4 ^b	0.68±0.08 ^b
Diabetes+Met	80±5 ^{a,b,c}	0.90±0.04 ^b
Control+Ap	40±4 ^{b,d}	0.78±0.05 ^b

Each group represents Mean±SEM of 6 animals. Significance at $P < 0.05$. a– compared with control; b– compared with diabetic control; c– compared with diabetes+Ap; d– compared with diabetes+Met; *Units: mg/dL.

No change was observed in the insulin receptor mRNA levels (Figure 2a). But, IR protein (Figure 2b) was significantly reduced

in the diabetic rats. *A. paniculata* was able to notably increase the IR level adeptly to that of metformin. In control+Ap group, the IR was not altered when compared to control. The IRS-1 mRNA (Figure 2c) and protein levels (Figure 2d)

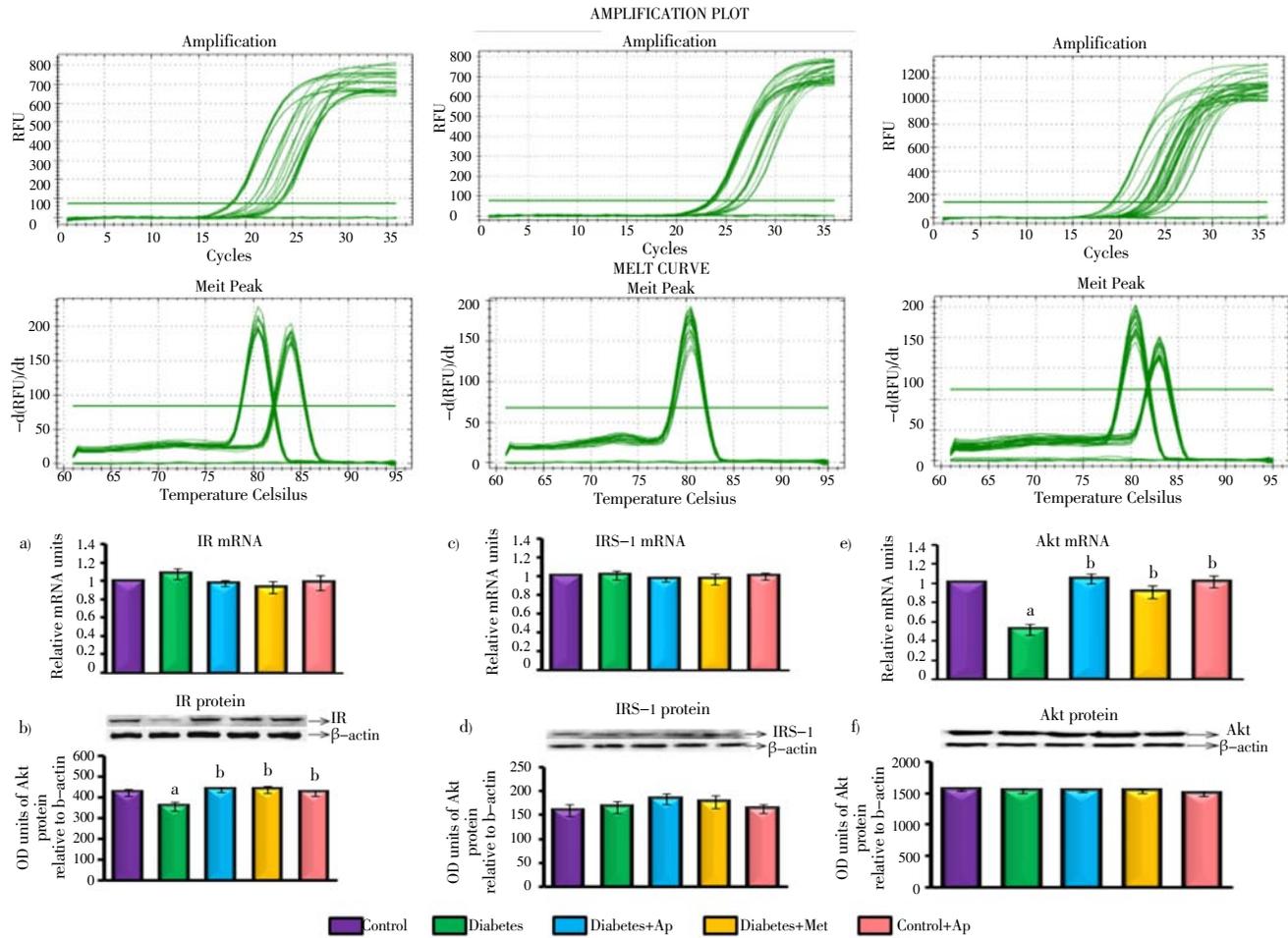


Figure 2. Effect of *A. paniculata* on mRNA and protein levels of IR, IRS-1 and Akt in liver of type-2 diabetic adult male rat. Each bar represents Mean±SEM of 6 animals. Significance at $P<0.05$, a- compared with control; b- compared with diabetic control.

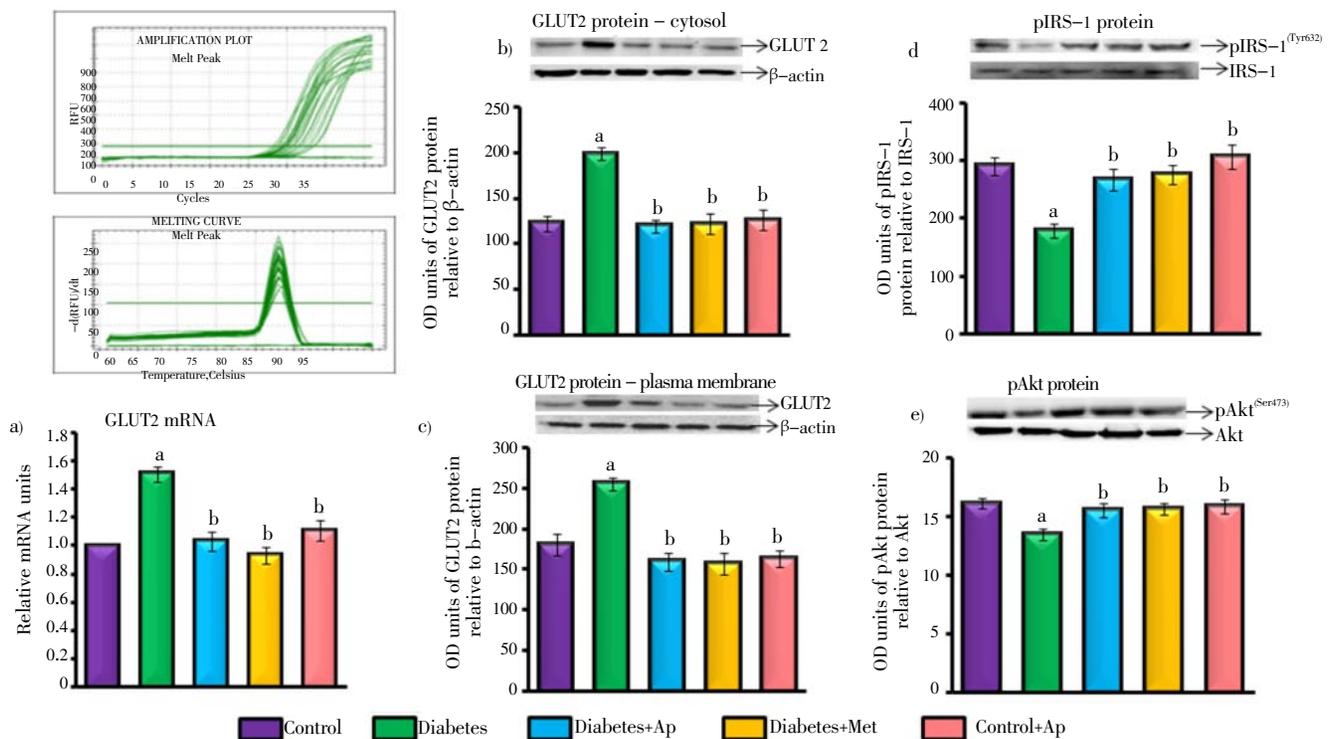


Figure 3. Effect of *A. paniculata* on mRNA, protein levels of GLUT2, pIRS-1 and Akt in liver of type-2 diabetic adult male rat. Each bar represents Mean±SEM of 6 animals. Significance at $P<0.05$, a- compared with control; b- compared with diabetic control.

were unaltered. The pIRS-1 (Figure 3d) was considerably reduced in the diabetic group and this was brought back to the normal range in the diabetes+Ap group.

A significant decrease was seen in the Akt mRNA levels (Figure 2e) in the diabetic group of liver. *A. paniculata* notably increased the Akt mRNA when compared to the diabetic group. No change was observed in the Akt protein levels (Figure 2f). The Akt serine phosphorylation (Figure 3e) was significantly decreased in the diabetic group. *A. paniculata* notably increased the Akt serine phosphorylation when compared to the diabetic group.

GLUT2 mRNA (Figure 3a) and protein levels [cytosolic (Figure 3b) and plasma membrane (Figure 3c)] were significantly increased in the diabetic group and *A. paniculata* ably restored it to control range.

4. Discussion

The fasting blood glucose was significantly increased in the high fat and sucrose-induced diabetic group compared to control while the *A. paniculata* treatment to diabetic group was able to significantly lower the blood glucose level similar to the metformin treated diabetic group. The antihyperglycemic effect of *A. paniculata* may be attributed to several mechanisms such as its ability to a) increase glucose uptake and oxidation in the peripheral tissues; b) impair absorption of glucose at the intestine (α -glucosidase inhibition)^[18]; c) enhance insulin sensitivity and thereby increasing the glucose transporters^[19]; d) control lipid metabolism thereby mending the putative inhibition of insulin signaling^[10,20]; e) scavenge the free radicals (antioxidant activity)^[21] which disrupt the plasma membrane integrity, resulting in decreased plasma membrane receptors or transporters necessary to signal and uptake glucose from the blood stream^[22]. In the present study, we have assessed the glucose uptake and oxidation in liver, one of the main organs necessary to maintain glucose homeostasis.

OGT test indicates impaired glucose tolerance, insulin resistance and type-2 diabetic condition. In the current investigation, OGT test reveals that high fat diet and sucrose impaired the glucose tolerance as evidenced by the sustained rise in blood glucose at 60, 120 and 180 min after glucose challenge when compared to control. In *A. paniculata* treated diabetic rats, the elevated blood glucose at 60 and 120 min was effectively brought down at 180 min. This is denotative of the ability of *A. paniculata* to tolerate the external glucose load effectively compared to that of metformin. Metformin is said to be antihyperglycemic rather than hypoglycemic^[23]. Likewise, *A. paniculata* treatment to normal control rats did not induce any change in blood glucose.

Despite an increase in serum insulin in the diabetic rat, the persistent hyperglycemic condition was observed in the

OGT test. This is indicative of insulin resistant condition. The antihyperglycemic activity of *A. paniculata* may also be attributed to its ability to decrease insulin as a result of improved insulin sensitivity.

High cholesterol, TG, LDL cholesterol and low HDL cholesterol, the characteristic dyslipidemia seen in insulin-resistant condition^[24] was also observed in the diabetic animals of the present study. *A. paniculata* being anti-triglyceridemic in nature ably prevents the dyslipidemia compared to that of metformin. In control+Ap group, no change was observed implying that the anti-triglyceridemic effect is prominent in dyslipidemic condition alone. High fat diet increased the serum liver and kidney marker enzymes which indicates the liver and kidney damage.

IR is the primary obligatory molecule necessary for insulin to translocate GLUT molecules from the cytosol to the plasma membrane. In the present study, IR protein was significantly reduced in the diabetic rats in spite of unaltered IR mRNA. Increased free radicals results in lipid peroxidation. The end product of lipid peroxidation forms adducts with the plasma membrane and this disrupts the membrane integrity thereby reducing the IR in the plasma membrane. *A. paniculata*, being a potent antioxidant inducer, was able to notably increase the IR level adeptly compared with metformin. In control+Ap group, the IR was not altered when compared to control.

The IRS-1 mRNA was unaltered but the Akt mRNA was decreased in the diabetic group. The decreased Akt mRNA in the diabetic group may be attributed to increased free fatty acids since, increased free fatty acids has been shown to decrease Akt mRNA^[25]. Although the IRS-1 and Akt levels were unaltered in all the groups, the phosphorylated form of both the proteins was considerably reduced in the diabetic group. Rapid onset of visceral obesity and fatty liver may occur with intake of a high-calorie diet that is high in fat and sucrose by increasing the expression of genes involved in the multiple steps of lipid accumulation and inflammation in liver and white adipose tissue^[26]. Accumulation of lipid in the liver has been proved to disrupt the insulin signaling by increasing the serine phosphorylation thereby decreasing the tyrosine phosphorylation^[27].

The mRNA of GLUT2 has increased significantly in the diabetic group. In T2DM, GLUT2 gene upregulation is augmented by binding of increased SREBP-1c^[28] to the GLUT2 promoter^[29]. Increased GLUT2 mRNA has reflected the increased GLUT2 cytosolic protein. In the plasma membrane fractions, GLUT2 was significantly augmented in the diabetic group. As insulin resistance condition is established in the present study, there is no regulation of glycogenolysis or gluconeogenesis. This results in increased glucose inside the liver. GLUT2 is increased at the plasma membrane to let this glucose out into the plasma. *A. paniculata* and metformin were able to elude this increase by evading the insulin resistance condition. In control+Ap

group, GLUT2 was unaltered compared to control.

The glucose uptake and oxidation in liver were clearly impaired in the high fat diet and sucrose fed diabetic rat, whereas *A. paniculata* was able to successfully restore it to the normal range. Decreased activation of the insulin receptor kinase may be at least partially responsible for insulin resistance in liver^[30]. Restoration of glucose uptake and oxidation in liver observed in the present study may be one of the mechanisms through which *A. paniculata* exerts its antihyperglycemic effect. In accordance with the present study, Sylow *et al.*^[31] reported that high fat feeding resulted in significant impairment in skeletal muscle glucose uptake.

In conclusion, results of the current study suggest that *A. paniculata* suppresses the rise in blood glucose putatively through controlling glucose uptake and oxidation, restoration of insulin signaling molecules in liver and decreasing the serum lipid profile.

The precise mechanism of action through which *A. paniculata* works at the molecular level in skeletal muscle is under progress in our laboratory.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgements

I would like to sincerely thank my friends and colleagues, G. Sharmila, S. Sharmila and L. Sheerin Banu for all their support and encouragement. The financial assistance by the University of Madras, Chennai, India (Award letter no: Ref: Official memo: URF/Fellowship/2009/1042, dated 10/12/09) in the form of a University Research Fellowship (URF) to Ms. A. Anne Williams is gratefully acknowledged. Infrastructural facilities by University Grants Commission–Special Assistance Programme–Departmental Research Support (UGC–SAP–DRS), University Grants Commission–Assisted Department (UGC–ASIST) and Department of Science and Technology–Fund for improvement of Science and Technology (DST–FIST) programs are also gratefully acknowledged.

Comments

Background

There is growing concerns about the escalating health threat that diabetes now poses. Diabetes is one of the major health and development challenges of the 21st century. According to the International Diabetes Federation, there are currently 371 million people living with diabetes and another 280 million are at high risk of developing the disease. Apart from conventional allopathic medicines,

traditional/alternative therapy plays a significant role in treating type–2 diabetes. *A. paniculata* has a wide range of medicinal properties and is being used since ancient times for the treatment of various diseases. The literature reports many instances where this plant is used to lower blood glucose level but the mechanism of action by which it accomplishes this feat is not clear. Besides finding the mode of action of the medicinal plants, it is also necessary to validate the usage of the plant.

Research frontiers

This study was performed in order to determine the molecular mechanism through which *A. paniculata* exhibits its antidiabetic activity. The parameters checked in this study are most appropriate to check the mode of action of *A. paniculata* step–by–step at one of the major sites of insulin action–liver.

Related reports

The available literature supports the glucose lowering effect of *A. paniculata* (Zhang and Tan, 2000; Hossain *et al.*, 2007; Fosola *et al.*, 2010). But, this paper deals with the molecular mechanism involved.

Innovations & breakthroughs

The induction of type–2 diabetes used in the present study is most relative to the type–2 diabetes condition in humans. *A. paniculata* is effective in maintaining the glucose and insulin level to that of control range which is the need of the hour in insulin resistant cases. Also *A. paniculata* seems to act at the molecular level, regulating the genes involved in insulin signaling.

Applications

The mechanism of action at the level of liver and serum parameters are analyzed and reported. Moreover, the minimal side–effects and cost effectiveness of this plant is promising as a potent anti–diabetic agent and may be tried out in clinical trials.

Peer review

Scientific validation of medicinal plants would help to popularize the same among the public. The present study has a clear approach in studying the mechanism of action in the antidiabetic effect of the plant, validating its antidiabetic property. The results are interesting and suggest that the antidiabetic activity of *A. paniculata* may be attributed to its antioxidant, antilipidemic and regulation of insulin signal transduction.

References

- [1] Rizza RA. Pathogenesis of fasting and postprandial hyperglycemia in type 2 diabetes: implications for therapy.

- Diabetes* 2010; **59**(11): 2697–2707.
- [2] Kishida K, Funahashi T, Shimomura I. Molecular mechanisms of diabetes and atherosclerosis: role of adiponectin. *Endocr Metab Immune Disord Drug Targets* 2012; **12**(2): 118–131.
- [3] Bamosa A, Elnour A, Kaatabl H, Al Meheithif A, Aleissa K, Al Almaie S. Zamzam water ameliorates oxidative stress and reduces hemoglobina1c in type 2 diabetic patients. *J Diabetes Metab* 2013; **4**: 249.
- [4] Israili ZH. Advances in the treatment of type 2 diabetes mellitus. *Am J Ther* 2011; **18**(2): 117–152.
- [5] Hosseyini ES, Kashani HH, Asadi MH. Mode of action of medicinal plants on diabetic disorders. *Life Sci J* 2012; **9**(4): 2776–2783.
- [6] Alexiou P, Demopoulos VJ. A diverse series of substituted benzenesulfonamides as aldose reductase inhibitors with antioxidant activity: design, synthesis, and in vitro activity. *J Med Chem* 2010; **53**(21): 7756–7766.
- [7] Alagesaboopathi C. Ethnomedicinal plants and their utilization by villagers in Kumaragiri hills of Salem district of Tamilnadu, India. *Afr J Tradit Complement Altern Med* 2009; **6**(3): 222–227.
- [8] Singh SK, Vishnoi R, Dhingra GK, Kishor K. Antibacterial activity of leaf extracts of some selected traditional medicinal plants of Uttarakhand, North East India. *J Appl Nat Sci* 2012; **4**(1): 47–50.
- [9] Noor A, Bansal VS, Vijayalakshmi MA. Current update on anti-diabetic biomolecules from key traditional Indian medicinal plants. *Curr Sci* 2013; **104**(6): 721–727.
- [10] Valdiani A, Kadir MA, Tan SG, Talei D, Abdullah MP, Nikzad S. Nain-e Havandi *Andrographis paniculata* present yesterday, absent today: a plenary review on underutilized herb of Iran's pharmaceutical plants. *Mol Biol Rep* 2012; **39**(5): 5409–5424.
- [11] Govindarajan M, Sivakumar R. Adulticidal and repellent properties of indigenous plant extracts against *Culex quinquefasciatus* and *Aedes aegypti* (Diptera: Culicidae). *Parasitol Res* 2012; **110**(5): 1607–1620.
- [12] Sukhdev SH, Dev D, Rakesh KV. *Compendium of medicinal and aromatic plants*. Vol. 2. New Delhi: ASIA; 2006, p. 50–148.
- [13] Dombrowski L, Roy D, Marcotte B, Marette A. A new procedure for the isolation of plasma membranes, T tubules, and internal membranes from skeletal muscle. *Am J Physiol* 1996; **270**(4 Pt1): E667–E676.
- [14] Nevado C, Valverde AM, Benito M. Role of insulin receptor in the regulation of glucose uptake in neonatal hepatocytes. *Endocrinology* 2006; **147**: 3709–3718.
- [15] Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. *J Biol Chem* 1951; **193**: 265–275.
- [16] Johnson AD, Turner PC. Epididymal carbohydrate metabolism. I. glucose-1-14-C and glucose-6-14-C metabolism by mouse, rat and rabbit tissues. *Comp Biochem Physiol A Comp Physiol* 1971; **39**(4): 599–604.
- [17] Kraft LA, Johnson AD. Epididymal carbohydrate metabolism. II. Substrates and pathway utilization of caput and cauda epididymal tissue from the rabbit, rat and mouse. *Comp Biochem Physiol B* 1972; **42**(3): 451–461.
- [18] Subramanian R, Asmawi MZ, Sadikun A. *In vitro* alpha-glucosidase and alpha-amylase enzyme inhibitory effects of *Andrographis paniculata* extract and andrographolide. *Acta Biochim Pol* 2008; **55**: 391–398.
- [19] Chaurasia A, Kharya MD, Sharma B, Roy P. Glucose metabolism and diabetogenic gene expression analysis of chloroform fraction of *Andrographis paniculata* (Nees) whole herb in diabetic albino mice. *J Complement Integr Med* 2012; **9**: doi: 10.1515/1553–3840.1632.
- [20] Nugroho AE, Andrie M, Warditiani NK, Siswanto E, Pramono S, Lukitaningsih E. Antidiabetic and antihyperlipidemic effect of *Andrographis paniculata* (Burm. f.) Nees and andrographolide in high-fructose-fat-fed rats. *Indian J Pharmacol* 2012; **44**(3): 377–381.
- [21] Singh D, Nigam R, Pandey V, Singh P. Status of lipid peroxidation and antioxidant enzymes in stress induced rats when treated with *Andrographis paniculata* extract and vitamin C. *Vet Pract* 2012; **13**(1): 60–62.
- [22] Rajesh P, Sathish S, Srinivasan C, Selvaraj J, Balasubramanian K. Phthalate is associated with insulin resistance in adipose tissue of male rat: role of antioxidant vitamins. *J Cell Biochem* 2013; **114**(3): 558–569.
- [23] Phung OJ, Scholle JM, Talwar M, Coleman CI. Effect of noninsulin antidiabetic drugs added to metformin therapy on glycemic control, weight gain, and hypoglycemia in type 2 diabetes. *JAMA* 2010; **303**(14): 1410–1418.
- [24] Reaven GM. Insulin resistance: the link between obesity and cardiovascular disease. *Med Clin North Am* 2011; **95**(5): 875–892.
- [25] Armoni M, Harel C, Bar-Yoseph F, Milo S, Karnieli E. Free fatty acids repress the GLUT4 gene expression in cardiac muscle via novel response elements. *J Biol Chem* 2005; **280**(41): 34786–34795.
- [26] Yang ZH, Miyahara H, Takeo J, Katayama M. Diet high in fat and sucrose induces rapid onset of obesity-related metabolic syndrome partly through rapid response of genes involved in lipogenesis, insulin signalling and inflammation in mice. *Diabetol Metab Syndr* 2012; **4**: 32.
- [27] Wang ZQ, Yu Y, Zhang XH, Ribnicky D, Cefalu WT. Ecdysterone enhances muscle insulin signaling by modulating acylcarnitine profile and mitochondrial oxidative phosphorylation complexes in mice fed a high-fat diet. *Diabetes* 2011; **60**(5): e16.
- [28] Chunga MJ, Choa SY, Bhuiyana MJ, Kima KH, Lee SJ. Anti-diabetic effects of lemon balm (*Melissa officinalis*) essential oil on glucose- and lipid-regulating enzymes in type 2 diabetic mice. *Br J Nutr* 2010; **104**(2): 180–188.
- [29] Pulla VK, Battu MB, Alvala M, Sriram D, Yogeewari P. Can targeting SIRT-1 to treat type 2 diabetes be a good strategy? A review. *Expert Opin Ther Targets* 2012; **16**(8): 819–832.
- [30] Samuel VT, Shulman GI. Mechanisms for insulin resistance: common threads and missing links. *Cell* 2012; **148**: 852–871.
- [31] Syłow L, Jensen TE, Kleinert M, Hojlund K, Kiens B, Wojtaszewski J, et al. Rac1 signaling is required for insulin-stimulated glucose uptake and is dysregulated in insulin resistant murine and human skeletal muscle. *Diabetes* 2013; **62**(6): 1865–1875.