Research Article

Aqueous Bark Extracts of Terminalia Arjuna Stimulates Insulin Release, Enhances Insulin Action and Inhibits Starch Digestion and Protein Glycation *in Vitro*

Heather A J Thomson, Opeolu O Ojo, Peter R Flatt and Yasser H A Abdel-Wahab*

School of Biomedical Sciences, University of Ulster, UK

***Corresponding author:** Yasser H A Abdel-Wahab, School of Biomedical Sciences, University of Ulster, Coleraine, BT52 1SA, Northern Ireland, UK, Tel: +442870324354; E-mail: y.abdel-wahab@ulster.ac.uk

Abstract

Scientific evidence for the effects of *Terminalia arjuna* on diabetes mellitus is lacking. This study investigated the anti-diabetic efficacy and mode of action of the bark extract of *Terminalia arjuna*. Insulin-release from BRIN-BD11 cells was assessed in the absence or presence of *T. arjuna* extracts and modulators of insulin-secretion. Insulin concentration and intracellular calcium ($[Ca^{2+}]_i$) were measured by radioimmunoassay and FLEXstation™ respectively. Adipocyte glucose-uptake with 3T3-L1 fibroblasts, starch digestion with α-amylase and protein glycation were assessed *in vitro*. *Terminalia arjuna* extract stimulated insulin-release alone ($p<0.001$), in combination with known modulators but not without extracellular Ca^{2+}. It increased intracellular calcium but had no effects on depolarised cells. Glucose-uptake was enhanced in the presence of *T. arjuna* ($P< 0.001$). At higher concentrations, the extract decreased starch digestion and inhibited protein glycation ($p<0.001$). *Terminalia arjuna* extract possesses antidiabetic potential and may provide new opportunities for the treatment of diabetes.

Keywords: *Terminalia arjuna;* Diabetes; Insulin; Glucose uptake.

Background

The 21st century has seen increasing globalization, industrialization, longevity and major changes in lifestyle throughout the world. Because of these changes, projections indicate that by 2025, more than 5% of the world population will have diabetes [1,2]. In addition to this rapid increase in incidence, challenges facing the treatment and management of diabetes include known inefficacies of currently available therapies. This has created the need for novel treatment strategies that offer more benefits to people suffering from type 2 diabetes, focusing on treatments that produce better glycaemic control, appetite regulation, blood pressure and lipid reduction and weight loss [3].

Before the discovery of insulin in 1922, dietary treatments and plant-based therapies were commonly used for diabetes management. Over several decades, a number of comprehensive reviews have documented the utility of higher plants for treating aspects of diabetes, also providing discussion of the botany, photochemistry, pharmacology, and toxicology of botanical agents [4-8]. Previous studies in our laboratory have also provided research evidence for the anti-diabetic potential of extracts of various plant species [9-11].

Terminalia arjuna is a large tree belonging to the family *Combretaceae,* (Roxb Wight Arn) and indigenous to India, Myanmar and Sri Lanka. The bark of *T. arjuna* has been used in Indian native Ayurvedic medicine for over three centuries, primarily as a cardiotonic [12]. The bark of *Terminalia arjuna* has been found to have therapeutic benefit for the treatment of cardiovascular disease [13]. Clinical research has indicated its usefulness in relieving angina

pain, and in the treatment of coronary artery disease, heart failure and possibly hypercholesterolemia [14,15]. Ragavan and Krishnakumari [12] provided evidence for the beneficial effects of *T. arjuna* bark extracts in relation to the treatment of diabetes, reporting a reduction in serum glucose as well as protection against the destruction of pancreatic beta cells and kidney damage in rats with alloxan-induced diabetes. However, data on the effects of extracts of *T. arjuna* on insulin secretion and the mechanism behind its anti-diabetic effects are lacking. The aim of the present study was to investigate actions of *Terminilia arjuna* bark extract on insulin secretion and glucose uptake at the cellular level. Furthermore, possible effects on protein glycation and starch digestion were examined *in vitro*.

Materials and Methods

Plant material

Dried bark of *Terminalia arjuna* was obtained from a commercial supplier, Cure Herbs Pvt, Delhi, India. A voucher specimen of the plant is available in the company. Bark was homogenised to a fine powder and stored in opaque screw-top jars at room temperature (20 °C ± 2 °C) until use. For *in vitro* work, a decoction was prepared by bringing 25 g/l (dry weight) of material to the boil in water and allowed to infuse over 15 minutes. The suspension was filtered (Whatman No.1 filter paper) and the volume adjusted so the final concentration was 25 g/l. Aliquots (1 ml) of the filtered plant solution was brought to dryness under vacuum (Savant Speed vac, Savant Instrumentation Incorp., NY, USA). Dried fractions were stored at −20 °C until required. Fractions were reconstituted in incubation buffer for subsequent experiments as required.

Insulin secretion

Insulin release was determined using monolayers of clonal pancreatic cells, BRIN-BD11 [16]. Cells were grown in RPMI-1640 tissue culture medium containing 11.1 mmol glucose/l, 10 % foetal calf serum and antibiotics (50,000 IU penicillin-streptomycin/l), and maintained at 37 °C in an atmosphere of 5 % CO_2 and 95 % air. Twenty-four hours prior to acute experiments, cells were harvested and seeded in 24 - well plates at a density of 1.0 x 10^5 cells per well. Following overnight attachment, culture medium was removed and cells were pre-incubated for 40 min at 37 °C with 1 mL of krebs ringer bicarbonate (KRB) buffer supplemented with 1.1 mM glucose and 1 % bovine serum albumin (BSA). Subsequent test incubations were performed for 20 min at 5.6 mM glucose using similar buffer supplemented with aqueous plant extract and the agents indicated in Figures. Samples were stored at –20 °C for subsequent insulin radioimmunoassay [17]. Cell viability was assessed by a modified neutral red assay as described previously [18].

Intracellular calcium ($[Ca^{2+}]_i$)

Changes in $[Ca^{2+}]_i$ were determined fluorimetrically [19] using monolayers of BRIN-BD11 cells. The fluorescent probes are internalised by living cells during pre-incubation and emit characteristic fluorescence in relation to changes in cellular $[Ca_{2+}]_i$. Cells were seeded into 96-well plates (black walls, clear bottom, Costar, Roskilde, Denmark) at a density of 1.0 x 10^5 viable cells per well and allowed to attach overnight in culture. The cells were washed once with KRB buffer (115 mM NaCl, 4.7 mM KCl, 1.28 mM $CaCl_2$, 1.2 mM KH_2PO_4, 1.2 mM $MgSO_4$, 10 mM $NaHCO_3$ and 0.1 % (w/v) bovine serum albumin, pH 7.4) supplemented with 5.6 mM glucose, 20 mM HEPES and 500 µM of probenecid. The cells were incubated at 37 °C, for 1 hour with Ca^{2+} assay kit (Molecular Devices, Sunnyvale, California, USA), prepared with the same washing buffer, to a final volume of 200 µL. Fluorimetric data were acquired using a Flex Station scanning fluorimeter with integrated fluid transfer workstation (Molecular Devices, Sunnyvale, California, USA). Excitation, emission and cut-off filter were set to 485 nm, 525 nm and 515 nm. The Flexstation was set to run for 10 minutes, collecting data at a 2.5 second interval (6 reads per well). Test solutions (50 µL at 5X concentration) were transferred at 60 seconds from start of readings at a rate of 78 mL/s.

Adipocyte differentiation and cellular glucose uptake

3T3-L1 fibroblasts obtained from the American Type Culture Collection ((ATCC) Virginia, USA), were used to determine glucose uptake [20]. Cells (passages 5-10) were seeded in 12-well plates at a density of 1.0 x 10^5 cells per well, maintained at 37°C ± 2 °C with 5 % CO_2 and fed every 2 days with DMEM supplemented with penicillin (50 U/mL), streptomycin (50 µl/mL) and foetal bovine serum (10 % v/v). Adipocyte differentiation was initiated as described in detail elsewhere by the addition of 1 µg/mL insulin, 0.5 mM IBMX and 0.25 µM dexamethosone [20]. Prior to acute tests, cells were incubated in serum free DMEM for 2-3 hours to establish basal glucose uptake. Cellular glucose uptake was determined for 15 min at 37 °C using KRB buffer supplemented with tritiated 2-deoxyglucose (0.5 µCi/well), 50 mM glucose, insulin and other test agents as indicated in the Figures. Hexose uptake was terminated after 5 minutes by three rapid washes with ice-cold PBS, after which cells were detached by the

addition of 0.1 % sodium dodecyl sulphate (SDS) and subsequently lysed. Scintillation fluid was added and mixed thoroughly to solubilise the cell suspension. Radioactivity was measured on a Wallac 1409 Scintillation Counter (Wallac, Turke, Finland).

Starch digestion

To assess *in vitro* starch digestion, 100 mg of soluble starch (Sigma-Aldrich, St. Louis, MO, USA) was dissolved in 3 ml of distilled water in the absence and presence of plant extract or acarbose 50 µg/ml (Bayer AG, Germany) as a positive control. 40 µl of 0.01 % heat stable α-amylase (from *Bacillus leicheniformis)*, Sigma-Aldrich, St. Louis, USA) was added to all tubes. After incubation at 80°C for 20 minutes, the mixture was diluted to 10 ml and 1 ml was incubated with 2 ml of 0.1 M sodium acetate buffer (pH 4.75) and 30 µL of 0.1 % amyloglucosidase from Rhizopus mold (Sigma-Aldrich, St. Louis, USA) for 30 minutes at 60 °C. Glucose released, was measured on the Analox GM9 glucose analyzer (Analox Instruments, London, UK).

Protein glycation

A simple *in vitro* system was employed to assess protein glycation based, using insulin as a model substrate [21]. In brief, 100 µl of human insulin (1 mg/ml) was incubated in 10 mM sodium phosphate buffer (pH 7.4) with 220 mM D-glucose, plant extract or aminoguanidine (positive control) for 24 h. Sodium cyanoborohydride was added and the reaction was stopped by addition of 0.5 M acetic acid. Glycated and non-glycated insulin were separated and quantified using reversed-phase high performance liquid chromatography [21].

Statistical analysis

All results are expressed as mean ± SEM for a given number of observations (n). Groups of data were compared statistically using one-way ANOVA test followed by Newman-Keul post hoc comparison for data sets with $p < 0.05$.

Results

Insulin secretion studies

Aqueous extract of *Terminalia arjuna* produced a dose-dependent increase in insulin secretion from BRIN-BD11 cells at 5.6 mM glucose (5 mg/ml and above, n=8, p<0.001, Figure 1A). Cell viability assessed by neutral red assay remained was unchanged with the increase in insulin release (Figure 1B). The extract significantly enhanced insulin secretion at lower (1.1 mM) and higher (16.7 mM) glucose concentrations, P<0.001, n=8), (Figure 2). The insulin secretory activity of the extract was partially inhibited by verapamil (50µM, 39%, P<0.01) and diazoxide (300µM, 34%, P<0.01), (Figure 2). Furthermore, insulin secretion was further enhanced (2-fold, p<0.001) in the presence of the plant extract (5mg/ml) and IBMX (100µM). The plant extract produced no significant enhancement of insulin secretion in the presence of depolarizing concentration of KCl (Figure 2). Insulin release elicited by glibenclamide (200µM) and tolbutamide (200µM) also increased by 130% (p<0.001) and 170% (p<0.001) respectively in the presence T. *arjuna*. The insulin secretary activity *Terminalia arjuna* was abolished in the absence calcium (Figure 3).

Intracellular calcium $[Ca^{2+}]_i$ studies

Aqueous *Terminalia arjuna* extract (5 mg/ml) induced a sharp rise followed by a sustained increase in $[Ca^{2+}]_i$ (Figure 4A). Addition
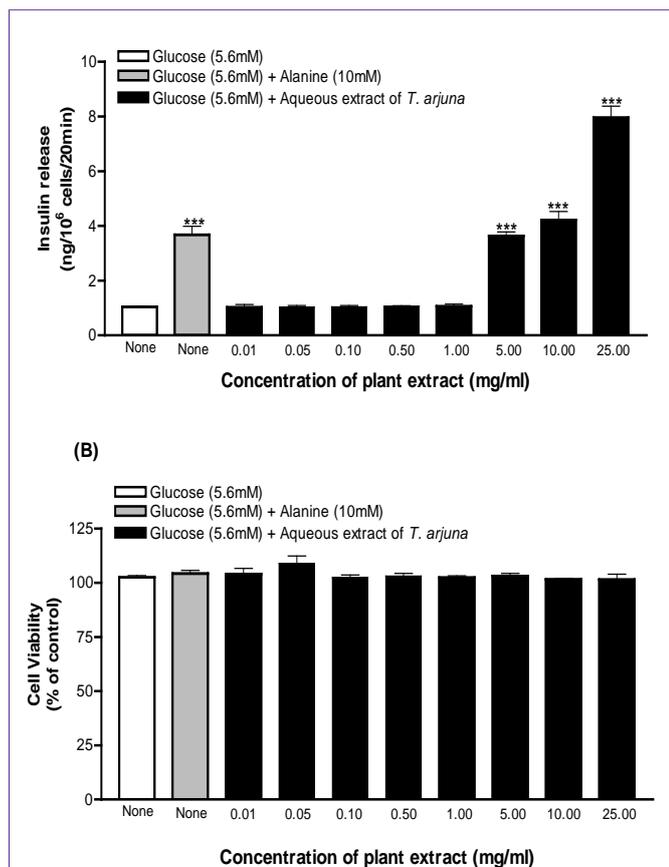
Figure 1: Dose-dependent effects of *Terminalia arjuna* on insulin secretion from BRIN: BD11 cells. BRIN-BD11 cells were incubated in KRB buffer supplemented with 5.6mM glucose in the presence or absence of increasing concentrations of plant extract for 20 min. Concentration of insulin and cell viability were measured in the supernatant retrieved after incubation period. Values are mean ± SEM with n = 8 for insulin and n = 6 for cell viability. ***P<0.001 compared to the control.
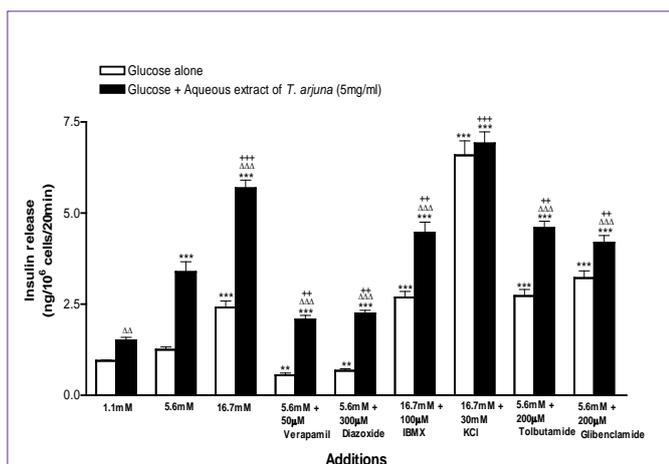

Figure 2: Modulation of *Terminalia arjuna* extract-induced insulin secretion by established stimulators and inhibitors of beta cell function. Values are mean ± SEM of 8 separate observations. **P<0.01, ***P<0.001 compared to glucose (control) in presence or absence of plant extract. ^ΔΔP<0.01, ^ΔΔΔP<0.001 compared to the respective incubations in absence of plant extract. ^++P<0.01, ^+++P<0.001 compared to 16.7mM glucose in presence or absence of plant extract.
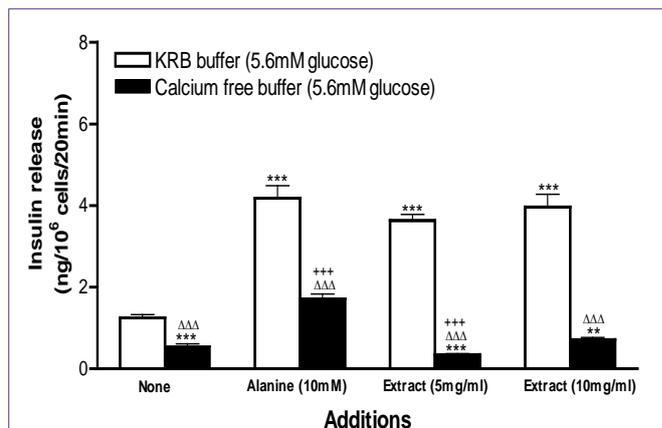

Figure 3: Effects of extracellular calcium on insulin release by *Terminalia arjuna*. BRIN-BD11 cells were incubated in calcium-containing or calcium-free KRB buffer supplemented with 5.6mM glucose in the presence or absence of plant extract for 20 min. Values are mean ± SEM of 8 separate observations. ***P<0.001 compared to 5.6mM glucose in the presence of Ca^{2+}. ^ΔΔΔP<0.001 compared to the respective concentration in the presence of Ca^{2+}. ^+++P<0.001 compared to 5.6mM glucose in the absence of Ca^{2+}.
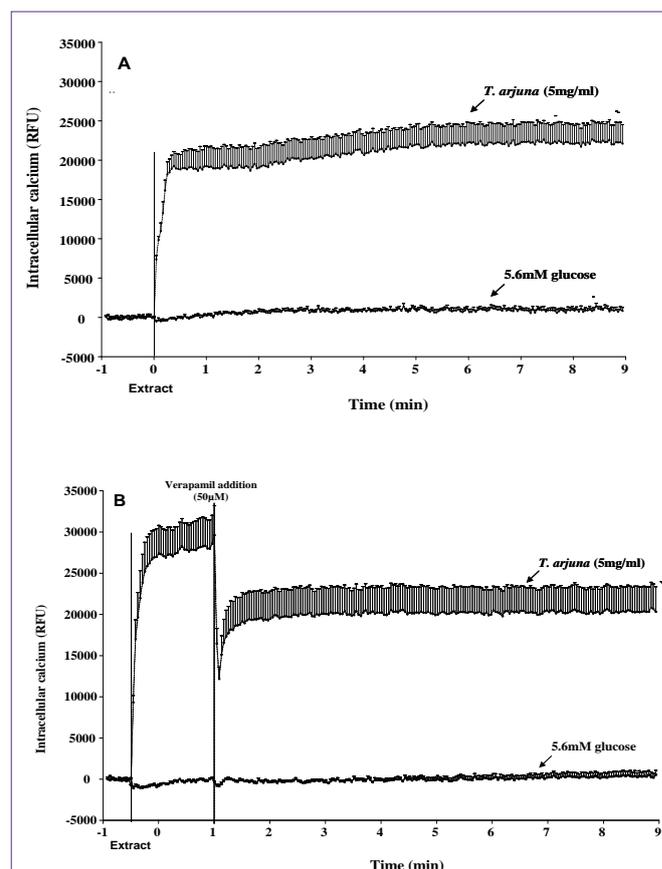

Figure 4: Effects of *Terminalia arjuna* on intracellular Ca^{2+} influx in the absence (A) and presence of Verapamil (B). Intracellular calcium concentration were measured by fluorescence spectroscopy in BRIN-BD11 cells incubated with KRB buffer containing FLIPR calcium dye and 5.6mM glucose in the presence or absence of plant material (5mg/ml) (Figure 4A). Verapamil (50μM) was added 60 seconds after the commencement of the experiment in Figure 4B. Data was collected every 1.25 seconds over a period of 540 seconds. Values are mean ± SEM of 6 separate observations.

of verapamil (50μM) resulted in a marked and sustained reduction in the sharp rise of $[Ca^{2+}]_i$ (Figure 4B).

Glucose uptake in vitro

Exposure of 3T3-L1 cells to aqueous extract of *Terminalia arjuna* produced a 178% increase in glucose uptake compared to control (p<0.01, Figure 5). This effect was greater than the effects of insulin alone (10^{-9}M, p<0.01, Figure 5). Concurrent incubation of the extract (5mg/ml) and insulin (10^{-9}M) resulted in 96% (p<0.01) increase in glucose uptake compared to insulin alone (Figure 5).

Starch digestion

In the presence of acarbose (1 mg/ml, positive control), enzymatic glucose liberation from starch was inhibited by 90% (P<0.001)

Figure 5: Effects of *Terminalia arjuna* extracts on 2-deoxy-D-[H³] glucose transport. Results are mean ± SEM of 4 separate observations. ***P<0.001 compared with incubations in the absence of insulin. +++P<0.001 compared to 10^{-9} M insulin alone. ΔP<0.05 compared to *Terminalia arjuna* incubations without insulin.

Figure 6: Effects of *Terminalia arjuna* extracts on starch digestion Soluble starch (100 mg) was dissolved in water in the absence or presence plant material or acarbose (50 μg/ml, positive control) and incubated at 80ºC for 20 minutes prior to measurement of the concentration glucose liberated. Results are mean ± SEM of 3 separate observations. ***P<0.001 compared to glucose liberated in absence of plant extract or acarbose.

compared to control. Aqueous extract of *T. arjuna* also significantly inhibited starch digestion at concentrations >10mg/ml. The highest inhibitory effect (74%, P<0.001) was observed at 50 mg/ml of the plant extract (Figure 6).

Protein glycation

Aqueous extract of Terminalia arjuna exerted a partial inhibition of insulin glycation (Figure 7). Over a range of concentrations tested (1-10 mg/ml), the plant extract significantly decreased protein glycation by 42% to 69% (p<0.001, Figure 7).

Discussion

Aqueous extract of *Terminalia arjuna*, stimulated insulin from BRIN-BD11 cells dose-dependently at concentrations ≥ 5 mg/ml. The stimulatory effects were not associated with beta cell cytotoxicity, confirming that the observed insulinotropic action was not due to simple leakage of insulin from the cells. Chelation of extracellular Ca^{2+} also inhibited the stimulatory effect of the plant extract, suggesting that Ca^{2+} plays an important role in the mode of action of the plant constituents. *T. arjuna* maintained its insulin-releasing effects; albeit at lower level, in the presence of verapamil (an inhibitor of voltage-gated Ca^{2+} channels) or diazoxide (K-$_{ATP}$ channels opener) [22]. This indicates that the insulinotropic effects may also involve an additional Ca^{2+}-independent pathway. The extract also enhanced the insulinotropic activity of sulphonylureas tested, consistent with a potentiation of the K$_{ATP}$-channel dependent activities of these secretagogues. This was confirmed by substantial rise of intracellular calcium ($[Ca^{2+}]_i$) in response to *T. arjuna* which was attenuated but not completely abolished by verapamil. This rise in $[Ca^{2+}]_i$ is probably a result of the opening of the voltage dependent calcium channels (VDCC) due to beta cell-depolarizing effects. The physiologic nature of this observation was confirmed by the attenuation of the increase in $[Ca^{2+}]_i$ by Ca^{2+} channel blockade with verapamil. The importance of plasma membrane action was also evidenced by no further stimulation of high secretion from beta cells depolarised by 30mM KCl.
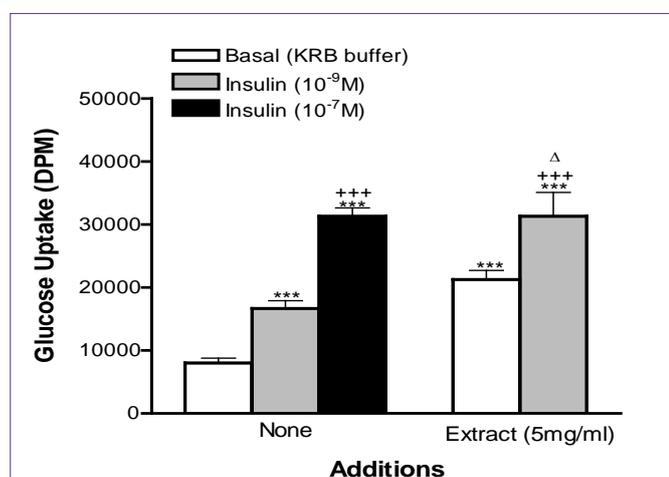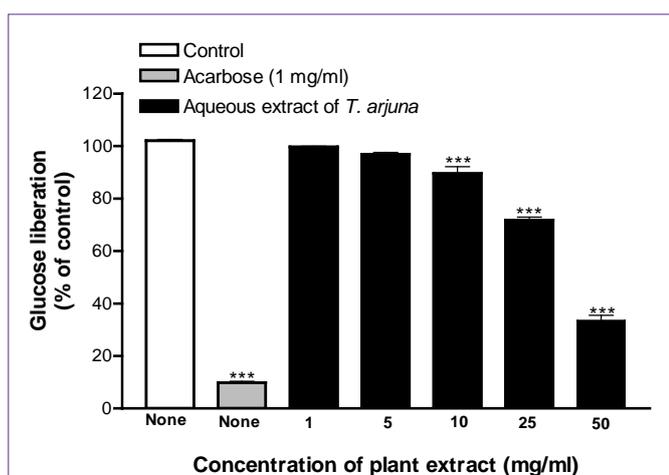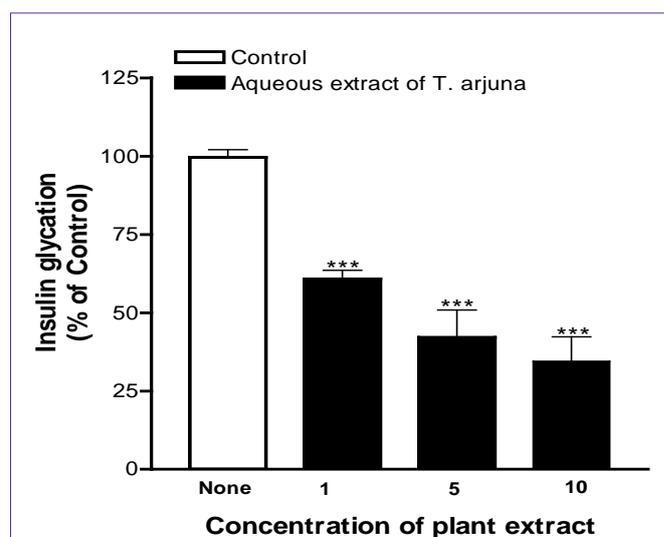
Figure 7: Effects of *Terminalia arjuna* extract on protein glycation. Results are mean ± SEM of 3 separate observations. **P<0.01, ***P<0.001 compared to glycation in the absence of plant extract.

In addition to the prominent beta cell stimulatory effects, aqueous extract of *Terminalia arjuna* enhanced cellular glucose transport in differentiated 3T3-L1 adipocytes. This effect was evident at lower concentration. The stimulatory action of insulin (10^{-9} M) was also augmented in the presence of the extract and the combined actions of the extract plus insulin exceeded the effects of either agent alone. As previously documented [15], the presence of trace elements in *Terminalia arjuna* bark extract may be one factor contributing to its ability to enhance insulin action [23,24].

Using a simple *in vitro* test consisting of the digestive enzymes α-amylase and α–glucosidase, the potential of *Terminalia arjuna* to retard starch digestion was evaluated by its effect on glucose liberation from starch. In this system, the established α-glucosidase inhibitor, acarbose, completely inhibited glucose liberation. In contrast, *Terminalia arjuna* extract produced a significant reduction in starch digestion at relatively higher concentrations of 10 mg/ml and above. Components of the extract responsible for this effect are unknown but the bark of *Terminalia arjuna* has been shown to contain high amounts of fiber [25] which may impede nutrient absorption *in vivo*. Ram et al. [14] demonstrated that bark extract of *T. arjuna* decreased deposition of fat in the liver and heart of rabbits.

In the final series of experiments, the ability of *Terminalia arjuna* aqueous extract to inhibit protein glycation was assessed using insulin as a model substrate [21]. The choice of insulin was based on the biological significance of glycated insulin with respect to pathophysiology of diabetes. It has been reported that glycated insulin exists *in vivo* and has reduced biological activity [26,27]. In this simple system, aqueous extract of *Terminalia arjuna*, significantly decreased glycation of insulin at concentrations ≥1mg/ml. Ethanolic extract of *Terminalia arjuna* has been reported to contain constituents such as tannins, flavonoids and glycosides which exhibit significant antioxidant properties [28]. Thus, the anti-glycation effects demonstrated in the present investigation may be a result of the antioxidative properties of some of its constituents.

Conclusion

In conclusion, aqueous bark extracts stimulated insulin secretion, enhanced insulin action, inhibited starch digestion and prevented protein glycation. The ability of plant constituents to influence these parameters *in vivo* depends entirely on soluble active principle(s) being absorbed via the gut. Further work is required to assess the ability of *Terminalia arjuna* to exert antidiabetic effects *in vivo*. Identification of the active components of the plant and assessment of *in vivo* activities are also critical to the possible development into new agents for diabetes therapy.

References

1. King H, Aubert RE, Herman WH. Global burden of diabetes 1995-2025: prevalence, numerical estimates and projections. Diabetes Care. 1998; 21: 1414-1431.

2. Krentz AJ, Bailey CJ. Type 2 Diabetes in Practice. London: Royal Society of Medicine Press Ltd; 2001.

3. Katsilambros N, Tentolouris N (2003). Type 2 diabetes: An overview. In: Textbook of Diabetes (Pickup JC, Williams G, eds), pp41-47. Oxford: Blackwell Science.

4. Swanston-Flatt SK, Day C, Bailey CJ, Flatt PR. Traditional plant treatments for diabetes. Studies in normal and streptozotocin diabetic mice. Diabetologia. 1990; 33: 462-464.

5. Atta-Ur-Rahman, Zaman K. Medicinal plants with hypoglycemic activity. J Ethnopharmacol. 1989; 26: 1-55.

6. Ivorra MD, Payá M, Villar A. A review of natural products and plants as potential antidiabetic drugs. J Ethnopharmacol. 1989; 27: 243-275.

7. Marles RJ, Farnsworth NR. Antidiabetic plants and their active constituents. Phytomedicine. 1995; 2: 137-189.

8. Yeh GY, Eisenberg DM, Kaptchuk TJ, Phillips RS. Systematic review of herbs and dietary supplements for glycemic control in diabetes. Diabetes Care. 2003; 26: 1277-1294.

9. Hannan JM, Marenah L, Ali L, Rokeya B, Flatt PR. Ocimum sanctum leaf extracts stimulate insulin secretion from perfused pancreas, isolated islets and clonal pancreatic beta-cells. J Endocrinol. 2006; 189: 127-136.

10. Gray AM, Flatt PR. Nature's own pharmacy: the diabetes perspective. Proc Nutr Soc. 1997; 56: 507-517.

11. Swanston-Flatt SK, Day C, Flatt PR, Gould BJ, Bailey CJ. Glycaemic effects of traditional European plant treatments for diabetes. Studies in normal and streptozotocin diabetic mice. Diabetes Res. 1989; 10: 69-73.

12. Ragavan B, Krishnakumari S. Effect of T. arjuna stem bark extract on histopathology of liver, kidney and pancreas of alloxan-induced diabetic rats. Afr J Biomed 2006; 9:189 -197.

13. Kumar DS, Prabhakar YS. On the ethnomedical significance of the Arjun tree, Terminalia arjuna (Roxb.) Wight & Arnot. J Ethnopharmacol. 1987; 20: 173-190.

14. Ram A, Lauria P, Gupta R, Kumar P, Sharma VN. Hypocholesterolaemic effects of Terminalia arjuna tree bark. J Ethnopharmacol. 1997; 55: 165-169.

15. Miller AL. Botanical influences on cardiovascular disease. Altern Med Rev. 1998; 3: 422-431.

16. McClenaghan NH, Barnett CR, Ah-Sing E, Abdel-Wahab YH, O'Harte FP. Characterization of a novel glucose-responsive insulin-secreting cell line, BRIN-BD11, produced by electrofusion. Diabetes. 1996; 45: 1132-1140.

17. Flatt PR, Bailey CJ. Abnormal plasma glucose and insulin responses in heterozygous lean (ob/+) mice. Diabetologia. 1981; 20: 573-577.

18. Mathews JN, Flatt PR, Abdel-Wahab YH. Asparagus adscendens (Shweta musali) stimulates insulin secretion, insulin action and inhibits starch digestion. Br J Nutr. 2006; 95: 576-581.

19. Miguel JC, Patterson S, Abdel-Wahab YH, Mathias PC, Flatt PR. Time-correlation between membrane depolarization and intracellular calcium in insulin secreting BRIN-BD11 cells: studies using FLIPR. Cell Calcium. 2004; 36: 43-50.

20. Frost SC, Lane MD. Evidence for the involvement of vicinal sulfhydryl groups in insulin-activated hexose transport by 3T3-L1 adipocytes. J Biol Chem. 1985; 260: 2646-2652.

21. O'Harte FP, Højrup P, Barnett CR, Flatt PR. Identification of the site of glycation of human insulin. Peptides. 1996; 17: 1323-1330.

22. Dunn CJ, Peters DH. Metformin. A review of its pharmacological properties and therapeutic use in non-insulin-dependent diabetes mellitus. Drugs. 1995; 49: 721-749.

23. Kar A, Choudhary BK, Bandyopadhyay NG. Preliminary studies on the inorganic constituents of some indigenous hypoglycaemic herbs on oral glucose tolerance test. J Ethnopharmacol. 1999; 64: 179-184.

24. Kar A, Choudhary BK, Bandyopadhyay NG Comparative evaluation of hypoglycaemic activity of some Indian medicinal plants in alloxan diabetic rats. J Ethnopharmacol. 2003; 84: 105-108.

25. Vaidya AB. Terminalia arjuna in cardiovascular therapy. J Assoc Physicians India. 1994; 42: 281-282.

26. Abdel-Wahab YH, O'Harte FP, Ratcliff H, McClenaghan NH, Barnett CR. Glycation of insulin in the islets of Langerhans of normal and diabetic animals. Diabetes. 1996; 45: 1489-1496.

27. Hunter SJ, Boyd AC, O'Harte FP, McKillop AM, Wiggam MI. Demonstration of glycated insulin in human diabetic plasma and decreased biological activity assessed by euglycemic-hyperinsulinemic clamp technique in humans. Diabetes. 2003; 52: 492-498.

28. Slobodan VJ, Steen S, Mihajio T, Budmir M, Michal GS. Flavonoids as antioxidants. J Am Chem Soc 1994; 116: 4846-4851

Citation: Thomson HAJ, Ojo OO, Flatt PR, Abdel-Wahab YHA. Aqueous Bark Extracts of Terminalia Arjuna Stimulates Insulin Release, Enhances Insulin Action and Inhibits Starch Digestion and Protein Glycation *in Vitro*. Austin J Endocrinol Diabetes. 2014;1(1): 1001.