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Emblica officinalis Stimulates the Secretion and Action of Insulin and Inhibits Starch Digestion and Protein Glycation *In vitro*

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Authors' contributions

This work was carried out in collaboration between all authors. Authors VK, PRF and YHAW designed the study and wrote protocol. Author VK evaluated biological analyses and performed the statistical analysis. All authors wrote first draft and managed samples for analyses. All authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

Aim: Medicinal, edible and aromatic plants and natural products have been used worldwide for the management of diabetes mellitus. The aim of this study was to investigate the efficacy and mode of action of *Emblica officinalis* Gaertn. (Phyllanthaceae) used traditionally for treatment of diabetes.
Study Design: Using multiple *In vitro* models; this study was designed to investigate the antidiabetes efficacy and mode of action of *E. officinalis.*
Place and Duration of Study: School of Biomedical Sciences**,** University of Ulster, 2001-2004
Results: *E. officinalis* aqueous extracts (AEs) stimulated basal insulin output and potentiated glucose-stimulated insulin secretion concentration-dependently in the clonal pancreatic beta cell line, BRIN-BD11 ($p<0.001$). The insulin secretory activity of plant extract was abolished in the absence of extracellular Ca^{2+} and by inhibitors of cellular Ca^{2+} uptake, diazoxide ($p<0.001$, $n=8$). Furthermore, the extract increased insulin secretion in depolarised cells and further augmented insulin secretion triggered by IBMX and tolbutamide. *E. officinalis* AE (1 mg/mL) displayed insulin mimetic activity (230%, $p<0.001$). Furthermore, it enhanced insulin-stimulated glucose transport in 3T3 L1

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adipocytes by 460% ($p<0.001$). *E. officinalis* augmented also synergistically ($p<0.001$) insulin action, when co-incubated with insulin sensitizers; metformin (2.4-fold), vanadate (4.9-fold), tungstate (4.8-fold) and molybdate (6-fold). At higher concentrations (0.5-5 mg/mL), the extract also produced 8-74% ($p<0.001$) decrease in enzymatic starch digestion *In vitro*. *E. officinalis* AEs (1-50 mg/mL) inhibited protein glycation 44-87% ($p<0.001$).

Conclusion: This study has revealed that water soluble bioactive principles in *E. officinalis* extract stimulate insulin secretion, enhance insulin action and inhibit both protein glycation and starch digestion. The former actions are dependent on the bioeffective component (s) in the plant being absorbed intact. Future work assessing the use of *Emblica officinalis* as adjunctive therapeutic nutraceutical or as a source of bioactive antidiabetic principles may provide new opportunities for the integrated management/prevention/reversal of diabetes.

Keywords: Emblica officinalis Gaertn; (Phyllanthaceae); insulin secretion; insulin action; starch digestion; peptide glycation.

1. INTRODUCTION

Diabetes mellitus is a chronic progressive syndrome, initially characterized by a loss of glucose homeostasis resulting from defects in insulin secretion and insulin action, both resulting in impaired metabolism of glucose and other energy yielding lipids and proteins [1]. It has recently been described as a global epidemic, as 385 million people have diabetes in 2013. It is projected that the worldwide prevalence is likely to increase to 592 million by 2035 [2]. Diabetes is typically associated with a host of co-morbidities, including cardiovascular disease, renal function, deterioration of vision and neuropathy. Modern diabetes drugs can successfully treat the symptoms but fail to suppress the progression of diabetes and its complications. Even in the 21st century, despite tremendous advances in the synthetic medicinal chemistry, plants are considered an integral part of the health care system in many countries. This phenomenon is based not only on the traditions of the folk medicine in different civilizations but also on the fact that medicinal plants have a leading position in drug discovery [3]. Derived from a prototypic molecule in a plant with a long history of medicinal use in medieval Europe is metformin. It exemplifies an efficacious drug, the development of which was based on the use of Goat's rue (*Galega officinalis*, French lilac), to treat diabetes. More so, a novel amino acid derivative, 4-hydroxyisoleucine, extracted from fenugreek (*Trigonella foenum graecum*) seeds has been shown to stimulate glucose dependent insulin release from isolated rat and human islets and to improve directly insulin sensitivity [4-6]. Furthermore, Fenugreek acts by delaying carbohydrate digestion and absorption and enhancing insulin action [6-8].

Emblica officinalis (amla) has multiple medicinal properties. It is used as a cardiotonic, cerebral and intestinal tonic in Ayurveda [9]. It was recognized for antipyretic and analgesic effects [10]. Its decoction was used for treating diarrhea, dysentery, cholera sores and pimples, mainly due to its antibacterial activities [11-12]. Fruits are used in the Ayurvedic medicine 'triphala' as one of the ingredients [13]. In clinical studies, *E. officinalis* was 85% effective in cases with hyperchlorhydria [14-15]. Some fruit active ingredients inhibit reverse transcriptase hence the HIV-1 replication [16]. The crude extract of *E. officinalis* was reported to counteract the hepatoxic and renotoxic effects of metals [17-18] and was recommended as a useful remedy for management of Alzheimer [19]. In addition, *E. officinalis* has remarkable antioxidant properties [20-23]. It proved to be effective for

hypercholesterolemia [24-25]. The hypolipidemic [26] and anti-atherosclerotic effects of *E. officinalis* were confirmed. *E. officinalis* has antiproliferative [27-28] and chemo-preventive anticancer properties [29-37]. Antidiabetic efficacies of *Emblica officinalis* were also reported [38-40]. Diverse and comprehensive appraisals of *E. officinalis* pharmacological and medicinal propensities are reported [41-47]. Chemical constituents present in different parts of the plant were intensively investigated [47-49]. The present study was undertaken to investigate the antidiabetic efficacies of *E. officinalis* Gaertn. (Phyllanthaceae) Aqueous Extracts (AEs) on insulin secretion and action at the cellular level. Furthermore, possible extrapancreatic effects of *E. officinalis* AEs on protein glycation and starch digestion bioassays were examined *in vitro*, to elucidate the speculated mechanisms responsible for its reported antihyperglycemic bioeffects.

2. MATERIALS AND METHODS

2.1 Cell lines, Chemicals and Biochemicals

3T3-L1 fibroblasts were obtained from the American Type Culture Collection ((ATCC) Virginia, USA). Filter paper no.1 (Whatman), vacuum dryer (Savant Speedvac, Savant Instrumentation Incorporation, NY (USA) were used in extract preparations. Wallac 1409 Scintillation Counter was from Wallac, Turke (Finland). Analox GM9 Glucose analyzer was from Analox Instruments (London, UK). Acarbose was obtained from Bayer AG (Germany). Unless stated otherwise, all other reagents were purchased from Sigma (Sigma-Aldrich, St. Louis, MO, USA).

2.2 Plant Material

Dried fruits of *Emblica officinalis* were procured from a commercial supplier in Delhi, India, during the winter season and available from Top-Op (Foods) Ltd (Stanmore, Middlesex, UK). Voucher specimens are preserved in Diabetes Research Group, School of Biomedical Sciences and University of Ulster**.** Fruits were homogenised to a fine powder and stored in opaque screw-top jars at room temperature (20±2°C) until use. For *In vitro* work, a decoction was prepared by bringing 25 g/L of material to the boil in water. Once boiling, the suspension was removed from the heat 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. 1 mL aliquots of the filtered plant solution were brought to dryness under vacuum (Savant Speedvac, 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.

2.3 Insulin Secretion

Insulin release was determined using monolayer of BRIN BD11 clonal pancreatic cells [50]. BRIN-BD11 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 preincubated 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 [51]. Cell viability was assessed using a modified neutral red assay as described previously [52].

2.4 Adipocyte Differentiation and Cellular Glucose Transport

3T3-L1 fibroblasts obtained from the American Type Culture Collection (ATCC) Virginia, USA, were used to determine glucose transport [53]. 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 fetal 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 [52]. Prior to acute tests, cells were incubated in serum free DMEM for 2-3 hours to establish basal glucose transport. Cellular glucose transport 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 (*E. officinalis* AEs, metformin, vanadate, tungstate, molybdate) as indicated in the figures. Hexose transport was terminated after 5 minutes by 3 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 to solubilised cell suspensions and mixed thoroughly. Radioactivity was measured on a Wallac 1409 Scintillation Counter (Wallac, Turke, Finland).

2.5 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 1000 µg/mL (Bayer AG, Germany) as a positive control. Then 40 µL of 0.01% heat stable α-amylase from *Bacillus leicheniformis*, Sigma-Aldrich, St. Louis, USA) was added. 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).

2.6 Protein Glycation

A simple *In vitro* system was employed to assess protein glycation based on the use of insulin as a model substrate [54]. 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 44mM (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 [54].

2.7 Statistical Analysis

All results are expressed as mean ± S.E.M (standard error of the mean) for the given number of observations (n). Groups of data were compared statistically using unpaired Student's t test. Results were considered significant if $p<0.05$ and highly significant if $p<0.01$ and $p<0.001$.

3. RESULTS

3.1 Effects of *E. officinalis* AEs on Insulin Secretion

E. officinalis AEs increased insulin release from BRIN BD11 cells significantly in a dose-dependent manner over the concentration gradient (0.01-25 mg/mL) in the presence of 5.6 mM glucose (Fig. 1). The minimum effective concentrations of *E. officinalis* were from 0.05 mg/mL and above (2.0-11.3 fold increased-insulin secretory response ($p<0.01$-0.001 vs. control wells), Fig.1). Cell viability remained unchanged (data not shown). *E. officinalis* AE (1 mg/mL) initiated/augmented insulin secretion in the absence (3.7-fold) and presence (2-fold) of 16.7 mM glucose highly significantly ($p<0.001$, Fig. 2) but was reduced 52% ($p<0.01$) by diazoxide.

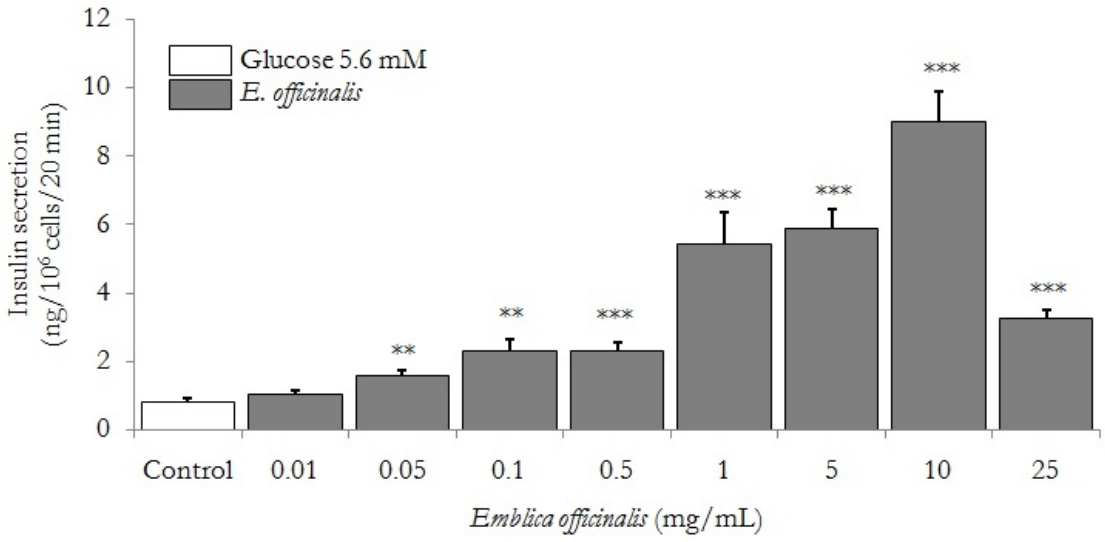

Fig. 1. **Effects of *E. officinalis* AEs on insulin release *In vitro***
*Values are mean ± SEM of 8 separate observations. **P<0.01 and ***P<0.001 compared to 5.6mM glucose alone (control)*

E. officinalis increased the insulin release in the presence of 16.7 mM glucose and 30 mM KCl (3.5-fold, $p<0.001$). In addition, it enhanced the insulin output in the presence of sulfonylurea tolbuatmide by 2.2-fold ($p<0.001$) (Fig. 2). The insulinotropic action of *E. officinalis* was significantly increased 3-fold ($p<0.001$) by 3-isobutyl-1-methylxanthine (IBMX), which increases β-cell cAMP levels. Interestingly, the insulin releasing effect of *E. officinalis* was increased 1.5-fold ($p<0.001$) by verapamil (Fig. 2). While in Ca^{2+} deprived incubations, and comparable to L-alanine, the insulin stimulatory effects ($p<0.001$) of *E. officinalis* (1 and 5 mg/mL) were reduced by 85% ($p<0.001$) and 36% ($p<0.001$), respectively in Fig. 3.

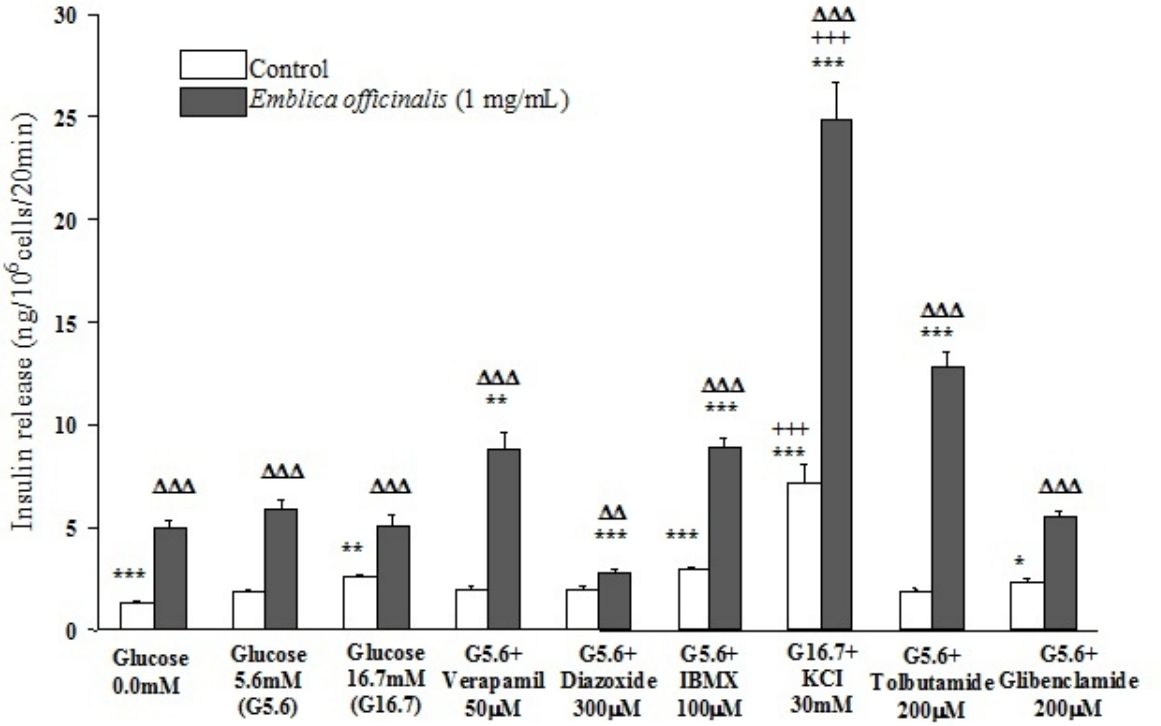

Fig. 2. *In vitro* Modulation of *E. officinalis* AEs-induced insulin secretion by established stimulators and inhibitors of beta cell function

*Values are mean ± SEM of 8 separate observations.*P<0.05, **P<0.01, ***P<0.001 compared to glucose (control) in presence or absence of plant extract. $^{\Delta}$P<0.05, $^{\Delta\Delta}$P<0.01 and $^{\Delta\Delta\Delta}$P<0.001 compared to the respective incubations in absence of plant extract. $^{+++}$P<0.001 compared to 16.7mM glucose in presence or absence of plant extract*

3.2 Effects of *E. officinalis* AEs on Insulin Action

E. officinalis AE (1 mg/mL) effected a 2.3 fold ($p<0.001$) increase in the basal 2-[^{3}H] deoxyglucose transport activity in 3T3-L1 adipocytes (Fig. 4) exceeding submaximal insulin action. *E. officinalis* 1 mg/mL co-incubated with 10^{-9}M insulin increased the sensitivity to insulin stimulation by 4.6-fold ($p<0.001$). Metformin (400 μM) doubled ($p<0.05$) the stimulatory effect of insulin (Fig. 5). Moreover, *E. officinalis*, 1 mg/mL, co-incubated with metformin and insulin, caused a 2.4-fold ($p<0.01$) potentiation in insulin stimulatory effect. Interestingly, vanadate (500 μM) did not augment the 1 nM insulin stimulated 2-[^{3}H] deoxyglucose transport (Fig. 6). However, comparable to maximal 10^{-6}M insulin effects, *E. officinalis* (1 mg/mL) co-supplemented with vanadate/insulin combination effected a highly significant 4.9-fold ($p<0.001$) increase of insulin stimulatory effects in 3T3L1 adipocytes. Tungstate (5mM) potentiated the stimulatory effects of insulin on 2-[^{3}H] deoxyglucose transport by 1.5-fold ($p<0.05$) (Fig. 7). *E. officinalis* (1 mg/mL) co-incubated with both 5mM tungstate/10^{-9} M insulin effected a profoundly significant ($p<0.001$) 4.8-fold augmentation of insulin evoked-2-[^{3}H] deoxyglucose transport, comparable to 10^{-6} M insulin action. Molybdate (30mM) significantly enhanced the 3T3-L1 fat cells sensitivity to insulin stimulating 2-[^{3}H] deoxyglucose uptake by 2.3-fold ($p<0.01$, Fig. 8). In the molybdate/insulin co-treatment wells, *E. officinalis* (1 mg/mL) affected synergistic ($p<0.001$) 6-fold increase of insulin-induced hexose transport, exceeding maximal insulin effects (the same figure).

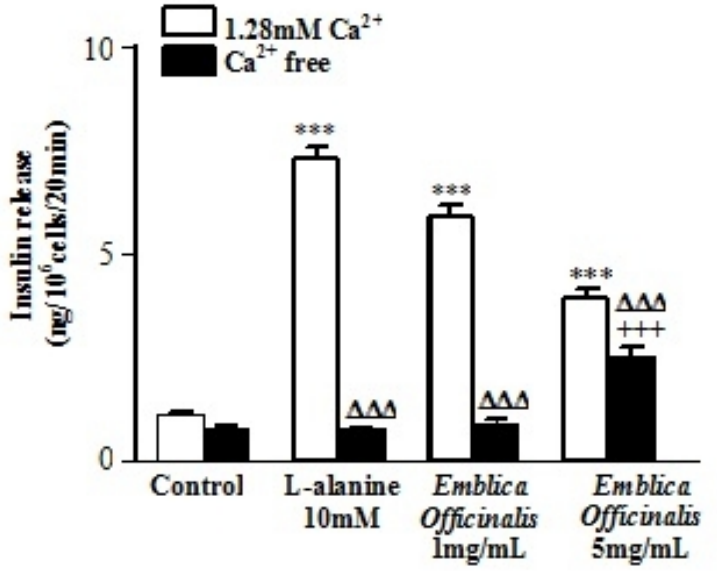

Fig. 3. *In vitro* Effects of *E. officinalis* AEs on insulin release in presence and absence of Ca^{2+}.

*Values are mean ± SEM of 8 separate observations. ***P<0.001 compared to 1.28 mM Ca^{2+}. $^{+++}$P<0.001 compared to respective Ca^{2+}free incubations. $^{\Delta\Delta\Delta}$P<0.001 compared to the respective compound in the presence of Ca^{2+}*

3.3 Effects of *E. officinalis* AEs on Starch Digestion

Using acarbose (1 mg/mL) as a positive control, glucose liberation from starch was inhibited by 98.9% (1.1±0.5% glucose liberated compared with 99.6±1.6% for control, P<0.001). The inhibition of starch digestion achieved by *E. officinalis* AEs at 5.0 mg/mL was 74% (p<0.001). The significant dose dependent (0.5-5.0 mg/mL, p<0.001 vs. control incubations) dual inhibitory effects of *E. officinalis* AEs (8-74%) on starch hydrolases, namely α-amylase and α-glucosidase, *In vitro* are demonstrated in Fig. 9.

3.4 Effects of *E. officinalis* AEs on Peptide Glycation

E. officinalis AEs (1-50 mg/mL) displayed a pronounced dose-related decrease in glycated insulin (44-87%, p<0.001 vs. control incubations, Fig. 10). This was comparable to the antiglycation effects (81% decrease in insulin glycation) of 44 mM aminoguanidine.

4. DISCUSSION

Clonal pancreatic β-BRIN BD11 cells are well characterized insulin secreting cell line with high insulin content, comparable with normal islets. It retains the physiological regulation of insulin secretion and is a good experimental model of normal and/or impaired insulin secretion studies and mechanisms of action of insulin secretagogues [55,56]. In this pancreatic cellular model, *E. officinalis* exerted dose dependent stimulatory effects on insulin secretion in the presence of 5.6 mM glucose with a maximum response at 10 mg/mL. A

large secretory response was obvious even in the absence of glucose. Given the linkage between cytosolic free Ca^{2+} increase and glucose metabolism in signaling insulin secretion [57], extracellular Ca^{2+} removal studies and toxicity testing using the modified neutral red assay further confirmed this regulated insulin output. Diazoxide, an antihypertensive drug that acts as K_{ATP} channel opener [58], inhibits the stimulatory action of SU by preventing closure of K_{ATP} channels [59] and in this study, the insulinotropic action of *E. officinalis*. The insulin releasing effect of tolbutamide was 7-fold potentiated synergistically with *E. officinalis* 1mg/mL. This is suggestive of a common site/mode of action of *E. officinalis* and SU drugs.

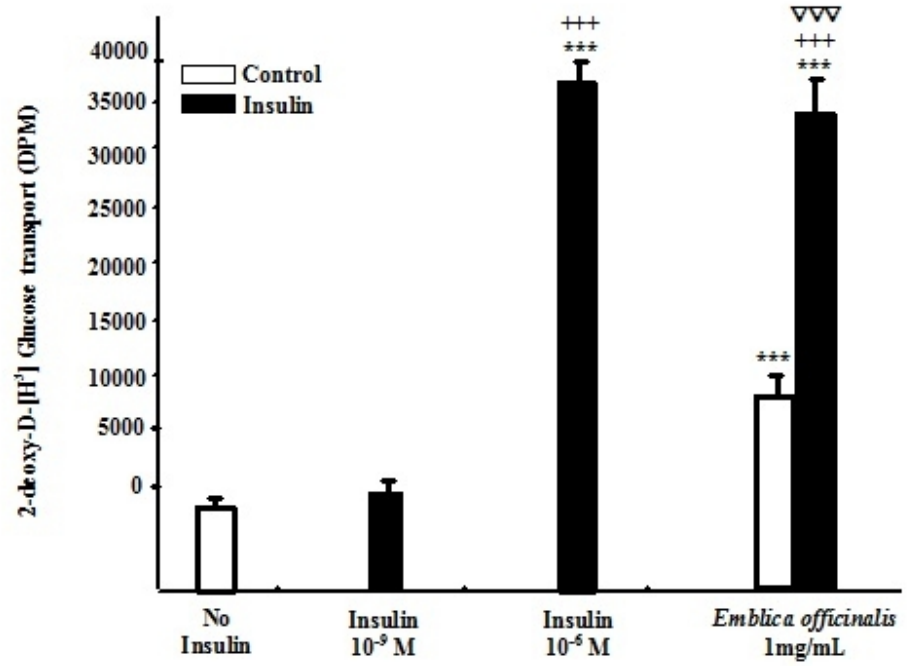

Fig. 4. *In vitro* Effects of *E. officinalis* AEs on 2-deoxy-D-[H^3] glucose transport

*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. $^{\nabla\nabla\nabla}$P<0.001 compared to E. officinalis incubations without insulin.*

On the other hand, the activity of *E. officinalis* was potentiated profoundly by the depolarizing combination of 30 mM KCl and 16.7 mM glucose, indicating similar K_{ATP} channel independent events. Verapamil, the prototype phenylalkylamine Ca^{2+} entry blocker, interacts preferentially with a binding site located within L type voltage-gated Ca^{2+} channels [60]. It inhibits sulfonylureas insulinotropic effects, but did not affect, interestingly, the physiologically regulated Ca^{2+} dependent insulin releasing action of *E. officinalis*. This suggests that *E. officinalis* possibly works through activating an additional secondary messenger that might increase the cytosolic Ca^{2+} from another pool causing insulin exocytosis [61]. Importantly, a combination of *E. officinalis* and the cAMP-phosphdiesterase inhibitor, IBMX, synergistically increased the β-cell secretion. In effect, this lends further weight to the potential discovery of novel insulinotropic entities in medicinal *E. officinalis*.

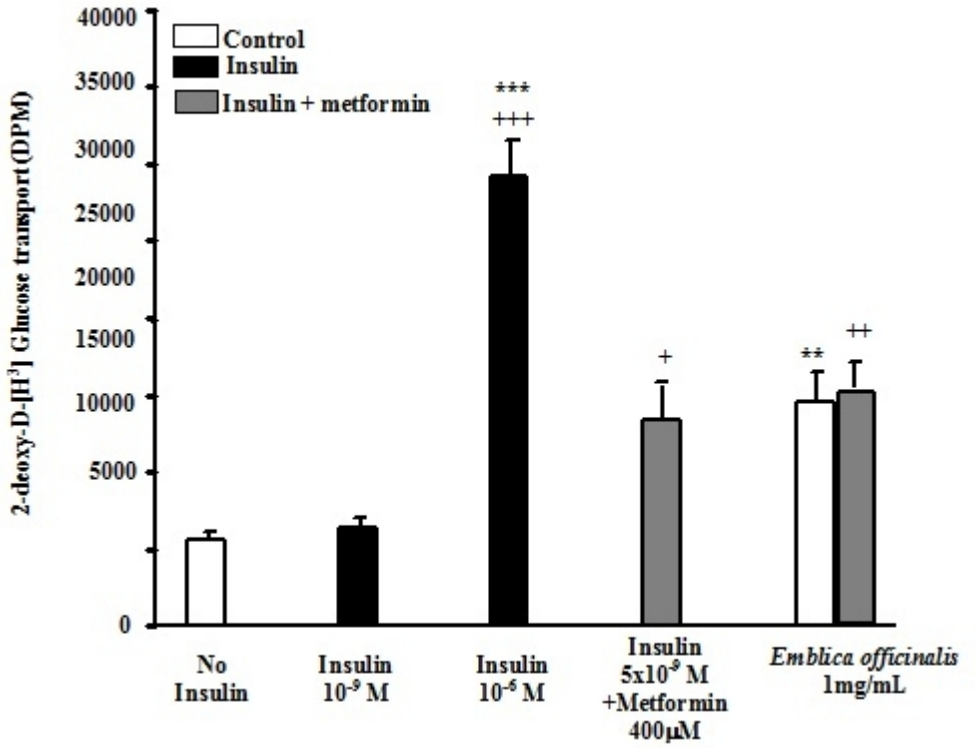

Fig. 5. *In vitro* Effects of *E. officinalis* AEs in combination with metformin on 2-deoxy-D-[H^3] glucose transport

*Results are mean ± SEM of 4 separate observations. ***P<0.01 and ***P<0.001 compared with incubations in the absence of insulin. $^{+}$P<0.05, $^{++}$P<0.01, $^{+++}$P<0.001 compared to 10^{-9} M insulin alone*

Insulin mimetic and potentiator of insulin action efficacies of *E. officinalis* were evident in differentiated 3T3L1 adipocytes acute incubation wells. *E. officinalis* augmented also synergistically insulin-mediated 2-[^{3}H] deoxyglucose transport activity when co-incubated with insulin sensitizers; metformin (2.4 fold), vanadate (4.9 fold), tungstate (4.8 fold) and molybdate (6 fold). These events were comparable or exceeding the maximal insulin actions, further highlighting the impressive insulin sensitizing properties of the plant. Thus, *E. officinalis* might be useful for alleviation of insulin resistance and in the study of the pathways leading to glucose utilization. In addition, future studies on plant extract bioactive principles are needed to assess the possible novelty of the mechanisms involved. The therapeutic effectiveness of the biguanide metformin requires the presence of insulin, improving some metabolic actions of insulin and extending additional effects that are independent of insulin [62]. Vanadate co-treatment with fenugreek seed powder reversed hyperglycaemia to control levels [63]. Tungstate and molybdate facilitated bio-effects in rat adipocytes only at high (millimolar) concentrations [64], with significant *In vivo* correlates via insulin like action [65].

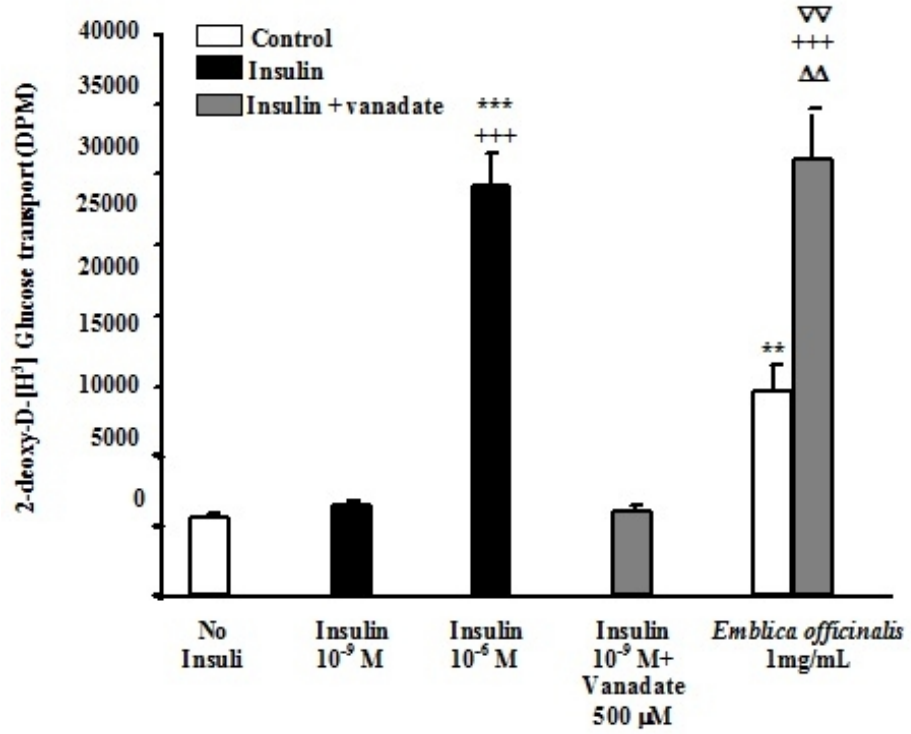

Fig. 6. *In vitro* Effects of *E. officinalis* AEs in combination with vanadate on 2-deoxy-D-[H^3] glucose transport

*Results are mean ± SEM of 4 separate observations. **P<0.01 and ***P<0.001 compared with incubations in the absence of insulin. $^{+++}$P<0.001 compared to 10^{-9} M insulin alone. $^{\Delta\Delta}$P<0.01 compared to plant incubations in absence of insulin. $^{\nabla\nabla}$P<0.01 compared to vanadate incubations in the presence of insulin.*

There has been much work done to elaborate on the etiology, prevention and treatment of diabetes related complications. Tight glucose control has been emphasized as being important in reducing diabetic microvascular disease in diabetes mellitus [66,67]. The glycation products -AGEs- are closely related to hyperglycemia and pathogenesis of diabetes related complications [68]. In addition, insulin glycation in β-cells can contribute to insulin resistance. This is based on the fact that glycated insulin has reduced biological activity [69-70]. The main AGE inhibitor discovered is aminoguanidine [71]. In this model of *in vitro* insulin glycation, *E. officinalis* exerted pronounced antiglycation effects. As oxidative stress in diabetes coexists with a reduction in the antioxidant status [72] and given the antiglycation - antioxidation correlation [73], the antioxidative efficacies of the *E.officinalis* extract can address its pronounced antiglycation effects [49,74-78]. This can be linked to its strong inhibition of the production of advanced glycosylated end products [79] in streptozotocin diabetic rats, thereby improving glucose metabolism. Other plausible antiglycation mechanisms can be considered, as *E. officinalis* tannoids proved effective in delaying diabetic cataract in rats via inhibition of aldose reductase activity and sorbitol formation, reversal of protein carbonyl content and subsequent aggregation and insolubilisation of lens proteins [80-81].

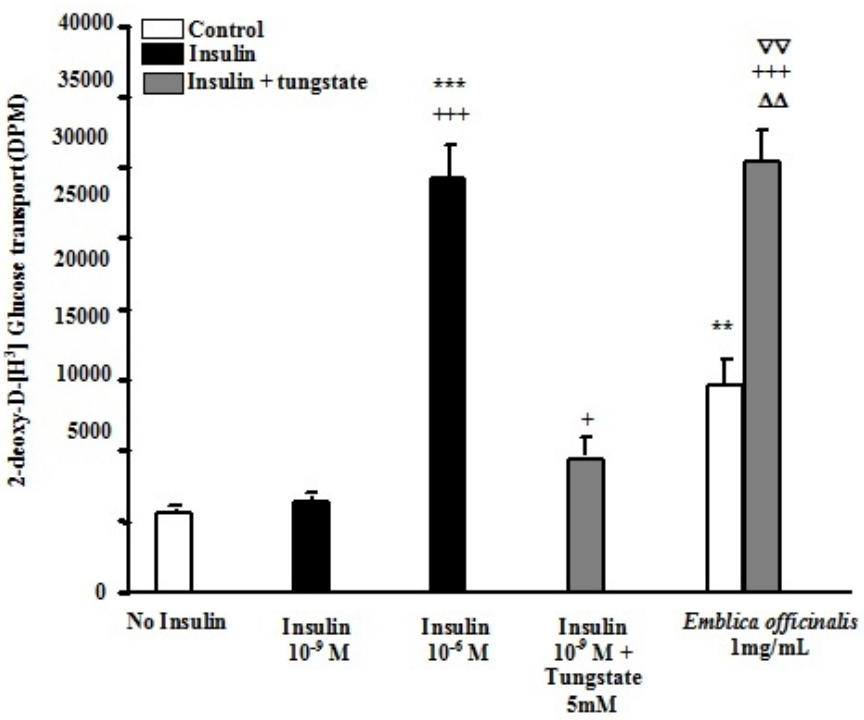

Fig. 7. *In vitro* Effects of *E. officinalis* AEs in combination with tungstate on 2-deoxy-D-[H^3] glucose transport

*Results are mean ± SEM of 4 separate observations. **P<0.01 and ***P<0.001 compared with incubations in the absence of insulin. $^{+}$P<0.05 and $^{+++}$P<0.001 compared to 10^{-9} M insulin alone. $^{\Delta\Delta}$P<0.01 compared to plant incubations in absence of insulin. $^{\nabla\nabla}$P<0.01 compared to tungstate incubations in the presence of insulin*

5. CONCLUSIONS

Succinctly, this study has highlighted that the *E. officinalis* AEs at pancreatic cellular levels, initiated stimulation of basal (no glucose) insulin release and potentiated glucose-evoked Ca^{2+}-regulated insulin output. In addition to insulin mimetic effect, *E. officinalis* (1 mg/mL) combined with 10^{-9} M insulin caused potentiation in insulin sensitivity in fat cells. Like acarbose, *E. officinalis* demonstrated highly significant dose dependent dual inhibitory effects on α-amylase and α-glucosidase. In insulin glycation system, *E. officinalis* extracts had a significant dose-related inhibition of insulin glycation. Future work is required to purify and characterize the water soluble active components of *E. officinalis* to bring forward potential novel agents for integrated diabetes management and prevention.

CONSENT

Not applicable.

ETHICAL APPROVAL

Not applicable.

ACKNOWLEDGEMENTS

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COMPETING INTERESTS

The authors declare that they have no conflict of interest concerning this article.

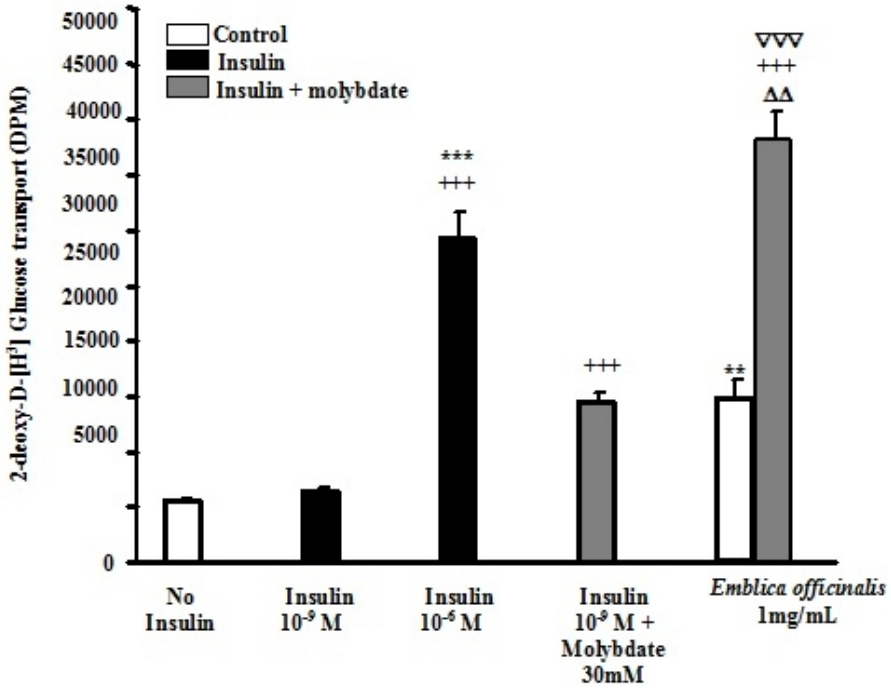

Fig. 8. *In vitro* Effects of *E. officinalis* AEs in combination with molybdate on 2-deoxy-D-[H^3] glucose transport.

*Results are mean ± SEM of 4 separate observations. **P<0.01 and ***P<0.001 compared with incubations in the absence of insulin. $^{+++}$P<0.001 compared to 10^{-9} M insulin alone. $^{\Delta\Delta}$P<0.01 compared to plant incubations in absence of insulin. $^{\nabla\nabla\nabla}$P<0.001 compared to molybdate incubations in presence of insulin*

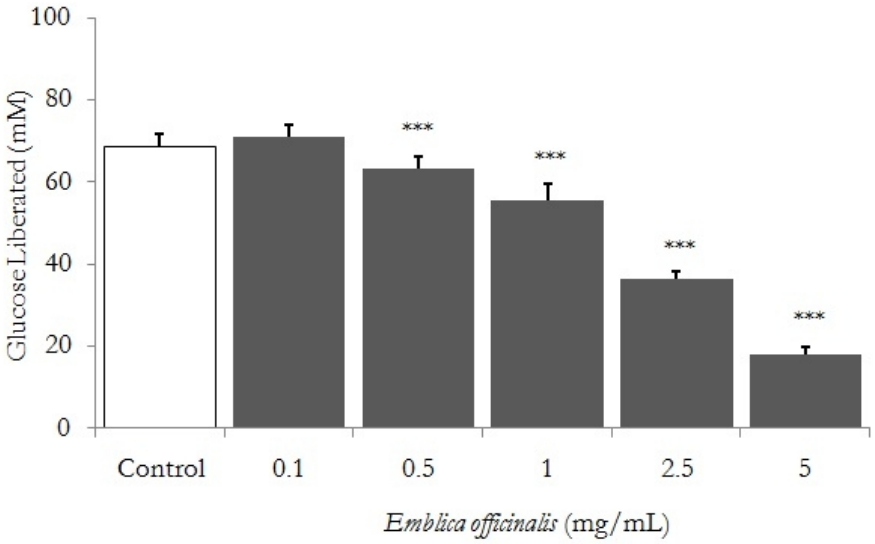

Fig. 9. Effects of *E. officinalis* AEs on starch digestion *In vitro*.
*Results are mean ± SEM of 3 separate observations. ***P<0.001 compared to glucose liberated in absence of plant extract*

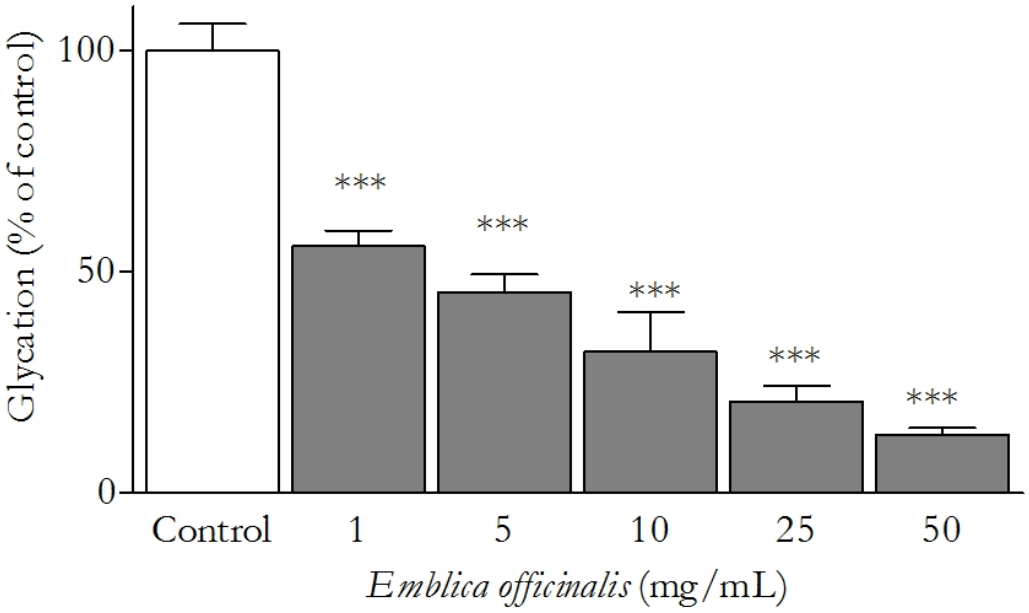

Fig. 10. Effects of *E. officinalis* AEs on protein glycation *In vitro*.
*Results are mean ± SEM of 3 separate observations. ***P<0.001 compared to glycation in the absence of plant extract*

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