Emblica officinalis stimulates the secretion and action of insulin and inhibits starch digestion and protein glycation in vitro

ABSTRACT

Aim: Traditional plant treatments have been used throughout the world for the therapy 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 Emblica officinalis.

Place and duration of Study: School of Biomedical Sciences, University of Ulster, 2012.

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. Emblica officinalis AE (1 mg/mL) displayed insulin mimetic activity (230%, P<0.001). Furthermore, it enhanced insulin-stimulated glucose transport in 3T3 L1 adipocytes by 460% (P<0.001). Emblica 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 starch digestion in vitro. E. officinalis AE (1-50 mg/mL) inhibited protein glycation 44-87% (P<0.001).

Conclusion: This study has revealed that 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, and it is projected that the worldwide prevalence is likely to increase to more than 300 million by the year 2025 [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 to 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 [4] and to improve directly insulin sensitivity [5, 6]. Furthermore, Fenugreek acts by delaying carbohydrate digestion and absorption and enhancing insulin action [6-8].

*Emblica officinalis* (amLa) has multiple extensively-reported medicinal properties. It is used as a cardiotonic, cerebral and intestinal tonic in Ayurveda [9], with antipyretic and analgesic activity [10]. Its decoction is used for treating diarrhoea, dysentery, cholera and applied to sores and pimples, mainly due to its antibacterial activities [11, 12]. Fruits are used in the Ayurvedic medicine ‘triplaha’ 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]. Chemical constituents present in different parts of the plant were intensively investigated [41, 42].

The present study was undertaken to investigate the antidiabetic efficacies of *Emblica officinalis* Gaertn. (Phyllanthaceae) AEs on insulin secretion and action at the cellular level. Furthermore, possible extrapancreatic effects 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 in/ from Top-Op (Foods) Ltd (Stanmore, Middlesex, UK). Voucher specimens are preserved in Diabetes Research Group, School of Biomedical Sciences, University of Ulster. Fruits was 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 Incorpor., NY, USA). Dried fractions were stored at –20 ºC until required. Fractions were reconstituted in incubation buffer for subsequent experiments as required.
Fig. 1. Effects of Emblica officinalis AEs on insulin release. Values are mean ± SEM of 8 separate observations. **P<0.01 and ***P<0.001 compared to 5.6mM glucose alone (control).

2.3. Insulin secretion

Insulin release was determined using monolayer of BRIN BD11 clonal pancreatic cells [43]. BRIN-BD11 cells were grown in RPMI-1640 tissue culture medium containing 11.1 mmol glucose/L, 10% fetal calf serum and antibiotics (50,000 IU penicillin-streptomycin/L), and maintained at 37 ºC in an atmosphere of 5% CO₂ 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 10⁵ cells per well. Following overnight attachment, culture medium was removed and cells were preincubated for 40 min at 37 ºC 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 ºC for subsequent insulin radioimmunoassay [44]. Cell viability was assessed using a modified neutral red assay as described previously [45].

2.4. 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 [46]. Cells (passages 5-10) were seeded in 12-well plates at a density of 1.0 X 10⁵ cells per well, maintained at 37ºC ± 2 ºC with 5% CO₂ 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 details elsewhere by the addition of 1 µg/mL insulin, 0.5 mM IBMX and 0.25 µM dexamethasone [45]. 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 ºC 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 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).
Fig. 2 Modulation of *E. officinalis* extract-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. ∆P<0.05, ∆∆P<0.01 and ∆∆∆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.

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. 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 ºC. 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 [47]. 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 [47].

2.7. Statistical analysis

All results are expressed as mean ± SEM 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. Insulin secretion studies

Emblica officinalis AEs increased insulin release from BRIN BD11 cells significantly in a dose-dependent manner over the concentration gradient (0.01-25mg/mL) in the presence of 5.6mM glucose (Fig. 1). The minimum effective concentration of Emblica officinalis was from 0.05 mg/mL and above (2.0-11.3 fold (p<0.01-0.001) increased-insulin secretory response, Fig. 1). Cell viability remained unchanged (data not shown). Extract (1 mg/mL) initiated/augmented insulin secretion in the absence (3.7-fold) and presence (2-fold) of 16.7mM glucose highly significantly (p<0.001, Fig. 2), but reduced 52% (p<0.01) by diazoxide. E. officinalis increased the insulin release in the presence of 16.7mM glucose and 30 mM KCl (3.5-fold, p<0.001), in addition, it enhanced the insulin output in the presence of sulfonylurea tolbutamide 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 Emblica officinalis was increased 1.5-fold (p<0.001) by verapamil (Fig. 2). While in Ca²⁺ deprived incubations, and comparable to L-alanine, the insulin stimulatory effects (p<0.001) of Emblica officinalis (1 and 5 mg/mL) were reduced by 85% (p<0.001) and 36% (p<0.001), respectively in Fig. 3.

![Fig. 3](image)

**Fig. 3** Effects of Emblica officinalis extract on insulin release in presence and absence of Ca²⁺. Values are mean ± SEM of 8 separate observations. ***P<0.001 compared to 1.28 mM Ca²⁺. +++P<0.001 compared to respective Ca²⁺ free incubations. ∆∆∆P<0.001 compared to the respective compound in the presence of Ca²⁺.

3.2. Insulin action

Emblica officinalis AE (1 mg/mL) affected a 2.3 fold (p<0.001) increase in the basal 2-[³H] deoxyglucose uptake activity in 3T3-L1 adipocytes (Fig. 4) exceeding submaximal insulin action. 1 mg/mL E. officinalis co-incubated with 10⁻⁶M insulin increased the sensitivity to insulin stimulation by 4.6-folds (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-[³H] deoxyglucose transport (Fig. 6). However, comparable to maximal 10⁻⁶M 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. Fig. 7 shows that tungstate (5mM) potentiated the stimulatory effects of insulin on 2-[³H] deoxyglucose transport by 1.5-fold (p<0.05). Emblica officinalis (1 mg/mL) co-incubated with 5mM tungstate/10⁻⁹ M insulin effected a profoundly significant (p<0.001) 4.8-fold augmentation of insulin evoked-2-[³H] deoxyglucose uptake, comparable to 10⁻⁶ M insulin actions. Molybdate (30mM) significantly enhanced the 3T3-L1 fat cells sensitivity to insulin stimulating 2-[³H] deoxyglucose uptake by 2.3-fold (p<0.01, Fig. 8). In
the molybdate/insulin co-treatment wells, the 1 mg/mL of *E. officinalis*, affected synergistic 
(p<0.001) 6-fold increase of insulin-induced hexose transport, exceeding maximal insulin effects.

**Fig. 4** Effects of *Embla officinalis* 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 △△△P<0.001 compared to E. 
officinalis incubations without insulin.

### 3.3. 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 *Embla officinalis* extract at 5.0 mg/mL was 74% 
(p<0.001). Fig. 9 demonstrates the significant (p<0.001) dose dependent (0.5-5.0 mg/mL) 
inhibitory effects of *E. officinalis* extracts (8-74%) on enzymatic starch digestion *in vitro*.

### 3.4. Glycation studies

*Embla officinalis* AEs (1-50 mg/mL) displayed a pronounced dose dependent decrease in 
glycated insulin (44-87%, Fig. 10). This was comparative 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 [48, 49]. In this pancreatic cellular model, 
*Embla officinalis* exerted dose dependent stimulatory effects on insulin secretion in the 
presence of 5.6 mM glucose with a maximum response at 10mg/mL. A large secretory response 
was obvious even in the absence of glucose. Given the linkage between cytosolic free Ca²⁺ 
increase and glucose metabolism in signalling insulin secretion [50], extracellular Ca²⁺ 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 [51], inhibits 
the stimulatory action of SU by preventing closure of K⁺ATP channels [52] and, in this study, the 
insulinotropic action of *E. officinalis*. The insulin releasing effect of tolbutamide was 7-fold
potentiated synergistically with 1mg/mL *E. officinalis*. This is suggestive of a common site/mode of action of *E. officinalis* and SU drugs.

![Graph](image)

**Fig. 5** Effects of *Emblica officinalis* extracts 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.

On the other hand, the activity of *E. officinalis* was potentiated profoundly by the depolarizing combination of 30mM KCl and 16.7mM glucose, indicating similar K\(_{\text{ATP}}\) 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 [53]. 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 [54]. Importantly, a combination of *E. officinalis* and the cAMP-phosphodiesterase inhibitor, IBMX, synergistically increased the \(\beta\)-cell secretion. In effect, this lends further weight to the potential discovery of novel insulinotropic entities in medicinal *Emblica officinalis*.

Insulin mimetic and potentiator of insulin action efficacies of *Emblica officinalis* were evident in differentiated 3T3L1 adipocytes acute incubation wells. *Emblica 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 sensitising 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 [55]. Vanadate co-treatment with
fenugreek seed powder reversed hyperglycaemia to control levels [56]. Tungstate and molybdate facilitated bio-effects in rat adipocytes only at high (millimolar) concentrations [57], with significant in vivo correlates via insulin like action [58].

Fig. 6 Effects of Emblica officinalis extracts in combination with vanadate on 2-deoxy-D-[H³] 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. ∆∆P<0.01 compared to plant incubations in absence of insulin. ++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 [59, 60]. The glycation products - AGEs- are closely related to hyperglycemia and pathogenesis of diabetes related complications [61]. 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 [62, 63]. The main AGE inhibitor discovered is aminoguanidine [64]. In this model of in vitro insulin glycation, Emblica officinalis exerted pronounced antiglycation effects. As oxidative stress in diabetes coexists with a reduction in the antioxidant status [65], and given the antiglycation - antioxidation correlation [66], the antioxidative efficacies of the E. officinalis extract can address its pronounced antiglycation effects [42, 67-71] and its strong inhibition of the production of advanced glycosylated end products [72] in streptozotocin diabetic rats, improving glucose metabolism. Other plausible antiglycation mechanisms can be considered, as Emblica 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 [73, 74].
Fig. 7  Effects of *Emblica officinalis* extracts 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. ΔΔΔP<0.01 compared to plant incubations in absence of insulin. ++P<0.01 compared to tungstate incubations in the presence of insulin.

5. CONCLUSIONS
Succinctly, this study has highlighted that the aqueous extract of *Emblica officinalis* at pancreatic cellular levels, initiated stimulation of basal (no glucose) insulin release and potentiated glucose-evoked 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, *Emblica officinalis* demonstrated highly significant dose dependent inhibitory effects on enzymatic starch digestion. In insulin glycation system, *Emblica officinalis* extracts had a significant dose-related inhibition of insulin glycation. Future work is required to purify and characterize the active components of *E. officinalis* to bring forward potential novel agents for integrated diabetes management.

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

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**Fig. 8** Effects of *Emblica officinalis* extracts in combination with molybdate on 2-deoxy-D-[H³] 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. ∆∆P<0.01 compared to plant incubations in absence of insulin. ∨∨∨P<0.001 compared to molybdate incubations in presence of insulin.

**Fig. 9** Effects of *Emblica officinalis* extracts on starch digestion. Results are mean ± SEM of 3 separate observations. ***P<0.001 compared to glucose liberated in absence of plant extract.
Fig. 10 Effects of *Emblica officinalis* extract on protein glycation. Results are mean ± SEM of 3 separate observations. ***P<0.001 compared to glycation in the absence of plant extract.

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