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In vitro Modulation of Pancreatic MIN6 Insulin Secretion and Proliferation and Extrapancreatic Glucose Absorption by *Paronychia argentea*, *Rheum ribes* and *Teucrium polium* Extracts

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ABSTRACT

The present study design investigated the effects of crude aqueous extracts (AE) of *Paronychia argentea* Lam.**,** *Rheum ribes* L. and *Teucrium polium* L., traditionally utilized in diabetes treatment in Jordan, on the pancreatic β-cell MIN6 proliferation and insulin secretion and extrapancreatic glucose diffusion. In an ascending order, *R. ribes, T. polium* and *P. argentea* concentrations induced a MIN6 monolayers expansion by respective 118-136%, 158-175% and 140-200% (P<0.001), thus exceeding GLP-1 (5 nM) pancreatic proliferative capacity. Like L-alanine (10 mM) insulinotropic efficacy and without exerting cytotoxicity, glucose stimulated insulin secretion was potentiated by AEs of *R. ribes* (373-736%, P<0.001) and *T. polium* (503-1190% P<0.001). *P. argentea* AE was inactive at used doses. The potent plants' insulin secretory bioactivities were abolished in the depleted Ca^{2+} conditions (P<0.001). Comparable to guar gum (50 mg/ml) diffusional hindrance in a simple dialysis model, *P. argentea* inhibited overnight glucose movement *in vitro* (by 38.1±1.9% AUC reductions, P<0.001), while *R. ribes* and *T. polium* AEs proved inactive. This *in vitro* evaluation has revealed that all three plants augmented β-cell expansion, *P. argentea* inhibited carbohydrate absorption and *R. ribes* and *T. polium* stimulated insulin secretion. These actions depend on their intact absorption *in vivo*. Future directives may assess the use of *P. argentea, R. ribes* and *T. polium* as new potential sources with functional properties for food or nutraceutical products or active leads into anti-diabetes pharmacotherapy.

Keywords: *Paronychia argentea* Lam, *Rheum ribes* L., *Teucrium polium* L., MIN6, glucose diffusion, Jordan.

INTRODUCTION

In accordance with the rising prevalence of diabetes mellitus (DM) worldwide, several studies have indicated that the incidence of type 2 DM and impaired fasting glycaemia in Jordan is increasing.[1-4] These observations have been confirmed by the International Diabetes Federation (IDF) data which stated that the current prevalence of DM in Jordan is at 10.1%. Among Middle Eastern and North Africa (MENA) countries this percentage indicates the ninth highest prevalence. [5]

On the other hand, the WHO estimated a high dependence of the world's population on plant remedies which has led to the renaissance of nutritional, clinical and scientific interest in the potential of plants for diabetes therapy.[6-9]

Type 2 DM is characterized by multiple defects in insulin action in tissues and defects in pancreatic insulin secretion, which eventually leads to the loss of pancreatic insulin-secreting cells. The treatment goals of T2DM have centered on using oral agents that promote insulin secretion, improving tissues' sensitivity to insulin or reducing the rate of carbohydrate absorption from the

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gastrointestinal tract [10] and retarding the development of diabetic complications. However, major changes in lifestyle factors should be addressed predominantly if the growing burden of diabetes is to be contained. There is still, however, an unmet need for the medicinal plants and phytopharmaceuticals with scientifically proven antidiabetic efficacy comparable to orthodox medicine. Due to the good acceptance of herbal drugs among the population, phytopharmaceuticals with demonstrated clinical efficacy could become a suitable alternative/complementary therapy to current medication for specific indications like the adjuvant treatment of diabetes. Locally, ethnopharmacological studies and surveys confirmed the appreciable prevalence of herbal use among patients with diabetes in Jordan.[11-13]

Given the rather remarkable and diverse potencies of *Paronychia argentea* Lam. (Caryophyllaceae) [14-16], *Rheum ribes* L. (Polygonaceae) [17-18] and *Teucrium pollium* L. (Labiatae/Lamiacae) [19-21] in reviewed literature, we conducted more detailed investigations to elucidate their antidiabetic pharmacological effects on cellular pancreatic insulin secretion and expansion. Furthermore, possible extrapancreatic effects on cell-free *in vitro* systems of carbohydrate absorption were examined.

RESULTS

Pancreatic β-cell viability and glucose-dependent modulation of insulin secretion by plants AE: Using the MIN6 cell line as the cellular model, the *in vitro* effects of the *Paronychia argentea*, *Rheum ribes* and *Teucrium polium* extracts on pancreatic β-cell proliferation and insulin secretion were investigated. MIN6 is a well-characterised insulin-secreting cell line with a higher insulin content than other β-cell lines, which retains the physiological regulation of insulin secretion and is an accepted good experimental model for the normal and/or impaired insulin secretion studies and for the evaluation of the mechanisms of action of insulin secretagogues.[22]

A colorimetric MTT assay was recruited to ascertain proliferative principles of chronic plant treatments. The gut hormone glucagon-like peptide-1 (GLP-1) agonists have been shown to stimulate the growth and differentiation of pancreatic cells, as well as to exert cytoprotective and antiapoptotic effects on β-cells.[23] Figures **1A-C** demonstrate that GLP-1 (5 nM [24]) promoted significant ($p<0.05$, n = 4) MIN6 proliferation by 140.35±1.12% in comparison to chronic basal (spontaneous) control incubations.

The MTT method revealed that 48h post seeding *P. argentea* extracts at doses 0.01, 0.05, 0.1 and 0.5 mg/ml expanded highly significantly the MIN6 monolayers by 140.4±3 – 200.2±2.5% ($p<0.001$ vs. control untreated cells, Figure **1A**). Higher concentrations proved, however unlikely, ineffective. Figure **1B** illustrates the highly marked MIN6 proliferative capacity of chronic treatments with *R. ribes* AE 0.1 mg/ml (117.7±6.8%, $p<0.05$), 10 mg/ml (124.3±4.9%, $p<0.001$) and 25 mg/ml (136±4.5%, $p<0.001$) vs. spontaneous chronic controls. Additionally, except for 5 mg/ml, highly substantial pancreatic expansive efficacies were ascribed to *T. polium* AE 0.01-10 mg/ml concentrations (157.6±4.5 - 174±9.3%, $p<0.001$ compared to basal untreated controls, Figure **1C**).

Furthermore, to evaluate the insulinotropic activity of plant extracts, a submaximal stimulatory glucose concentration (5.6 mM) was used in the acute incubations. L-alanine 10 mM – a well described and tested insulin secretagogue [7, 8] was used as a positive control and it augmented substantially GSIS in MIN6 by 163.7±18.9-178.5±17.9% ($p<0.05$) following 1h-incubations, compared to untreated controls (Figures **2A-C**). *Paronychia argentea* AE doses seemed to antagonise GSIS in MIN6 treatment wells compared to controls, with substantial % reductions ranging 30.81±4.91 – 68.36±3.02%($p<0.05$-0.001, Figure **2A**). Apart from the 25 mg/ml dose, MIN6 cell viability was unaffected, negating against plant-inflected cytotoxicity. Exceedingly superior to L-alanine, nevertheless, *Rheum ribes* crude AE at doses 0.01, 0.1, 0.5, 1, 10 and 25 mg/ml augmented the GSIS in pancreatic MIN6 highly markedly by 735.9±99.4, 469.9±87.9, 609.8±51.7, 372.5±71.4, 461.4±39.4 and 398.1±90.3%, respectively ($p<0.001$, Figure **2B)**. In similar lines of performance, *Teucrium*

polium AE concentrations of 0.01, 0.1, 1, 10 and 25 mg/ml enhanced pancreatic GSIS by respective 700±136, 1189±124, 679±21, 503±26 and 1933±184% highly significantly ($p<0.001$, Figure **2C**). Exceptionally, cell viability as checked by an MTT assay over a 1h-incubation with the potent plants AE was unchanged at the concentration ranges tested.

Ca^{2+} dependency of the insulinotropic effect of potent plants AE: Changes in β-cell cytosolic Ca^{2+} concentrations, whether by an influx of extracellular Ca^{2+} or by release of Ca^{2+} from intracellular stores, are thought to be a primary trigger for the initiation of insulin release. Figures **2B-C** illustrate that the marked insulinotropic trend of L-alanine was highly significantly (54.9±8.6%, $p<0.001$) abolished in Ca^{2+} depleted KRH, as compared to corresponding Ca^{2+} free glucose-only control wells. Comparable to L-alanine pancreatic physiology, Figure **2B** demonstrates that the Ca^{2+} free-KRH buffer has markedly reduced *R. ribes*-provoked MIN6 insulin output to respective 153±25.2, 86.3±7.5, 89.8±50, 107.4±31, 174.7±39.2 and 88.3±23% ($p<0.05$-0.001). Similarly, with *T. polium* effective insulinogenic concentrations, pancreatic GSIS in 1h- Ca^{2+} depleted-treatments was decreased substantially to respective 145±40, 172±21, 171±49, 204±40 and 622±72% ($p<0.01$-0.001, Figure **2C)**. Consistently, these observations suggest that *R. Ribes* and *T. polium* AEs potentiating of pancreatic GSIS is critically dependent on Ca^{2+} influx from extracellular pools.

Extrapancreatic modulation of glucose movement in vitro by plants AEs: Using the diffusion model described, the mean AUCs (24h glucose curve) for the viscous water-soluble gel-forming guar gum (10, 25 and 50 mg/ml) were decreased dependently and highly significantly by respective 7.0±0.5% ($p<0.05$), 20.5±6.4% ($p<0.001$) and 30.8±2.5% ($p<0.001$) (n=3, Figure **3**) compared to the overnight negative control. The efficacy of guar as a classical positive control has been elsewhere detailed.[25] Effectively comparable to guar, *Paronychia argentea* AE (50 mg/ml) only substantially retarded AUC for a 24h glucose efflux *in vitro* by 38.1±1.9% ($p<0.001$ versus the basal controls, n=3, Figure **3**). Lower concentrations proved ineffective. Also, *R. ribes* and *T. polium* extracts lacked any marked glucose diffusional hindrances in an external solution across the dialysis membrane (with respective 6.1±2.7% and 6.4±3.1% AUC reductions, $p>0.05$, Figure **3**).

Phytochemical analysis: The results of the TLC screening of the selected plants species are given in Table 1. Flavonoids and phenolics were identified in all tested plants, while the presence of alkaloids could be hardly detected in any of the three (the same table). Except for *T. polium*, none seemed to have terpenoids. Also, only *P. argentea* and *T. polium* had coumarins (the same table).

DISCUSSION

In Jordan, there is an appreciable prevalence of herbal use among patients with diabetes.[13] Pancreatic β-cells secrete insulin in response to elevated glucose to maintain blood glucose homeostasis. Defects in β-cell insulin secretion lead to hyperglycaemia and development of type 2 diabetes.[26] The distal events underlying stimulus – secretion coupling of insulin secretion have been well characterised. The function of the secretory machinery is mainly regulated by changes in the electrical activity of β-cell ion channels. Glucose uptake by β-cell enhances ATP production, followed by the closure of ATP gated K^+ channels leading to membrane depolarisation and subsequent opening of the voltage gated Ca^{2+} channels (VGCC). In this sequential glucose–stimulated insulin secretion, the influx of Ca^{2+} through VGCC and resultant increase in intracellular Ca^{2+} initiate exocytosis.[27-28] Acutely depleted Ca^{2+} conditions manifested a reduction in the exocytotic machinery, which was partially accountable for the impairments in effective plants potentiating of regulated secretory response.

*Rheum ribe*s, (rhubarb) as an edible vegetable, can be consumed for medicinal purposes.[29] The insulin releasing activity of three extracts of different polarity from *R. ribes* was illustrated *in vitro*, further evoking a significant hypoglycaemic effect in fasting healthy mice over 24 h periods.[30] The hypoglycaemic effect in alloxan-diabetic

animals is mentioned [17] along with antioxidant activity.[18] Here we clearly demonstrated the glucose-dependent insulinotropic effects of *R. ribes* AEs in mouse pancreatic MIN6 cells. The insulin secretory response to *R. ribes* was effectively minimised by the complete Ca^{2+} removal from extracellular pools, hence implying the association of depolarising β-cells and stimulating Ca^{2+} influx with the important role of *R. ribes* in augmenting an endogenously regulated insulin output. With a reported striking similarity to the trends of acarbose, *R. ribes* aqueous extract showed significant α-amylase and α-glucosidase inhibitory principles.[31] Despite the lack of glucose diffusional impediment properties, *R. ribes* AE impressively evoked pancreatic and extrapancreatic efficacies which offer a prospective therapeutic approach for the treatment of type 2 diabetes.

Locally, *Teucrium polium* was classified amongst the medicinal herbs widely used in Jordan with a low antioxidative capacity.[19] Elsewhere, *T. polium* lacked any appreciable plant-based retardation or delay of carbohydrate digestion [31] or absorption, as of our current findings. Nevertheless, *T. polium* fed-diabetic rats experienced significant reductions in serum glucose levels [20] via plausible *T. polium* flavonoids-mediated protection for pancreatic islets against exposure to STZ.[32-33] Especially important, the molybdate combination with *T. polium* significantly improved cultured rat islets insulin-secretory function.[34] Additionally, *T. polium* ethyl acetate fraction, owning to its antioxidant content, suppressed the formation of advanced glycation end products and protein glycoxidation *in vitro*.[35] In light of our study, without compromising cellular integrity, *T. polium* had the insulin secretory superiority (almost 1900% pancreatic physiology enhancement versus control wells) amongst the effective extracts. Changes in β-cell cytosolic Ca^{2+} concentrations, whether by an influx of extracellular Ca^{2+} or by release of Ca^{2+} from intracellular stores, are thought to be a primary trigger for the initiation of insulin release. Our observations demonstrated that the inhibition of extracellular Ca^{2+} influx perturbed the substantial *T. polium* induced GSIS in pancreatic MIN6 acute incubations. Collectively, the substantial extrapancreatic activity further complements the pancreatic activities of *T. polium* advocating the plant as a potential candidate with diabetes pharmacological modulation qualities. For future prospects, additional studies of the pancreatic modulatory activity of the potent plant components are needed to assess the possible novelty of the secretory mechanisms involved.

Among the main causes of diabetes is an absolute or relative decline in the number of functional β-cells. The preservation/restoration of β-cells mass is considered as one of the most promising therapeutic possibilities in the management of DM.[36] GLP-1 agonists exert their preservative/improving role of β-cell numbers as well as the blockade of diabetes progression.[23] Optimally, diabetes-regenerative therapeutics are fundamentally based on the significant enhancement of the self-duplication capacity of pre-existing β-cells rather than pluripotent stem cell differentiation.[37] Hence, intensive testing of plants inducing the pancreatic β-cell expansion will allow the emergence of safe and efficient cell replacement therapies. In this realm, *Paronychia argentea* chronic culturing wells maximally expanded pancreatic monolayers by 2 folds, followed by *Teucrium polium* (1.7 folds), and *Rheum ribes*, came in third, promoting the proliferation of the mouse β-cell line by 1.4 folds post - 48h treatment. Noticeably, the both *in vivo* reports on liver regenerative changes [38] as well as the significant increases in pancreatic islets numbers [39] in STZ-diabetic rats on *T. polium* AEs were confirmed by our demonstrated *T. polium* evoked pancreatic proliferative principles *in vitro*. Thus, the entire set of investigated plants offer a potentially promising avenue for treatment of β-cells demise in diabetes. To further explore the plant proliferative mechanism of action, diverse cellular processes warrant analyses. Moreover, application of a similar approach to human β-cells may demonstrate these plants' significant contribution to human β-cells proliferation.

In the present study, a simple *in vitro* dialysis-based model was used to investigate glucose absorption hindrance potency of *P. argentea* AE. The validity of this

model allows the *in vitro* investigation of the postprandial serum glucose lowering mechanism of aqueous plant extracts. Previously reported, *P. argentea* AE lacked any appreciable anti-α-amylase or anti-α-glucosidase effectiveness. Nevertheless, its doses of 250 and 500 mg/Kg b.wt decreased significantly the plasma sugar increments 90 min following glucose loading in normal fasting rats.[40] The effects of inhibiting markedly the glucose movement by *P. argentea* extracts can offer a plausible justification in this aspect. Most notably, published research suggests a direct relationship between a plant's ability to inhibit glucose absorption and the viscosity of its constituent soluble polysaccharides.[41-42] Fibre containing foods modify both the digestive and absorptive process.[42] Taken together, *P. argentea* represent potential alternatives that may be useful for improving glycaemia control via restricting postprandial glucose absorptions, and hence, allowing flexibility in meal planning in type 2 diabetes.

In conclusion, our data indicated that crude AEs of *R. ribes and T. polium* stimulated insulin secretion acutely. Interestingly, cellular viability was not affected by the effective plants concentrations, confirming that their insulinotropic effects were not due to simple leakage of insulin from the cells. Furthermore, depletion of extracellular Ca^{2+} inhibited the hormone secretion stimulatory effects, suggesting the importance of Ca^{2+} uptake in the mode of action of the active plants' constituents. This, in turn, implies that the insulin secretory response could be mediated by changes in K^{+} conductance at the MIN6 cell membrane. Also, all three plants augmented β-cell mass expansion in chronic culture conditions. Moreover, *P. argentea* inhibited extrapancreatic carbohydrate absorption. Further chronic investigation is required to validate their use prior to clinical implementation as therapeutic agents for improvements in diabetes. In our previous studies, the safety of these plants in doses up to 100 mg/ml was proved (unpublished data). Therefore, these findings support the notion that they may represent potentially useful sources with functional properties for nutraceutical products. Taken together, the obtained results also underline the possible health benefits associated with their consumption, thereby qualifying for discovery of new orally active antidiabetic therapeutics.

EXPERIMENTAL

Chemicals and biochemicals: Dulbecco Modified Eagle Medium (DMEM) containing 25 mM glucose was purchased from Invitrogen and the ELISA jumbo kit for rat high insulin was from ALPCO (USA). A MTT assay kit was purchased from Promega (USA). The assays were performed according to the manufacturer's instruction. Unless stated otherwise, all reagents and chemicals were obtained from Sigma (Dorset, UK). Dialysis tubing Spectra/Por® 7 Biotech Regenerated Cellulose (RC) membranes, MWCO 2000, were purchased from Spectrum Europe B.V, Breda, Netherlands. Falcon tube 50ml was obtained from Iwaki Scitech Div, Japan. Coated analytical thin layer chromatography (TLC) plates were procured from Merck, USA. A shaking incubator was from LabTech®, Daihan LabTech Co., LTD. (Korea). D (+) glucose was procured from Riedel-deHaen, Seize (Germany). Glucose GOD-PAP kit was obtained from BioLabo Reagents, France. For filtration of the water extracts, No.5 filter paper (Whatman, USA) was used. In UV determinations, a UV-VIS spectrophotometer from SpectroScan 80D (UK) was used.

Plant material: Fresh aerial parts of *P. argentea* [2 CARY-FMJ] and *T. pollium* [12 LABI-FMJ] were collected from the Greater Amman area and from Zai, 50km north of Amman in spring 2009. Dried subterranean parts (roots/rhizomes) of *R. ribes* [2 POLY-FMJ] were purchased from herbalist shops in Amman. Collectively, all plant materials were taxonomically identified by Prof. Barakat Abu Irmaileh, Faculty of Agriculture – University of Jordan. Voucher specimens were deposited in the Department of Pharmaceutical Sciences, Faculty of Pharmacy – University of Jordan. All collected fresh plant samples were air dried at room temperature and coarsely powdered.

Preparation of the aqueous extracts (AEs): AEs were prepared by refluxing each 10 g of the dried coarsely powdered plant material with 100 ml tap water

for 15 min. The extracts were kept overnight and filtered twice through filter paper. The volume of the filtered solution was increased to 100 ml with tap water to obtain 10% (equivalent to 100 mg/1ml) crude aqueous solutions.[18]

MIN6 cell culture: As described earlier by Miyazaki et al.,[43] pancreatic β-cells MIN6 (passage 39-45) were maintained in DMEM containing 15% foetal bovine serum (FBS), 100U/ml penicillin, 100µg/ml streptomycin, 100µg/ml L-glutamate and 5µl/L β-mercaptoethanol in a 37°C humidified atmosphere with 95% air and 5% CO_2. The culture medium was changed every 48 - 72h.

Insulin secretion static incubation experiment: Glucose stimulated insulin secretion (GSIS) from MIN6 cells was determined using a static incubation protocol. MIN6 were cultured in 96-well plates at density 50,000cell/well until 80% confluent. On the day of the experiment after removing the growth medium, the cells were washed with phosphate-buffered saline (PBS). Cells were pre-incubated for 1h at 37°C in 5% CO_2 in a HEPES-balanced Krebs-Ringer phosphate buffer (KRH) composed of (in mM) 129 NaCl, 5 $NaHCO_3$, 4.8 KCl, 1.2 KH_2PO_4, 1.2 $MgSO_4$, 10 HEPES, 2.5 $CaCl_2$ and 0.1% BSA (pH 7.4, NaOH) supplemented with 1.1 mM glucose. The incubation medium was removed, and the cells were washed once in glucose free KRH. Subsequent test incubations in 5.6 mM glucose - KRH alone (untreated negative control) or supplemented with appropriate treatments (L-alanine 10 mM or plants AE 0.01-25 mg/ml, n=4 observations) were performed for another 1h. Cell viability was assessed post 1h - acute incubations using MTT assays. There was a modification to this standard protocol. When investigating the effects of extracellular Ca^{2+}-free incubations on pancreatic insulin secretion, 2.5 mM $CaCl_2$ was removed from the KRH buffer preparations, so that cells were incubated in a Ca^{2+}-free KRH in the same panel of GSIS studies described previously.[44] For all experiments, the incubation medium was collected and stored at -20 °C for a subsequent ELISA (ALPCO) determination of the amount of secreted insulin.

Cell viability and proliferation assay: With minor modification on Smirin et al.[45] methodology, cell viability and proliferation were assessed by an MTT kit. MIN6 cells were subcultured on 96-well plates at a concentration of 10,000 cells/well in growth medium containing GLP-1 (5 nM) or different concentrations of plants AEs (0.01-25 mg/ml; n=4). After 48h of incubation, the effects of the plant extracts on cell viability were evaluated according to the kit's manufacturer protocol.

Glucose movement in vitro: A 0.22M glucose solution (in 0.15 M NaCl) was added to dialysis tubing (10cm x 11mm). This was maintained wet, as drying may result in an unrecoverable collapse of the pore structure. The tubing was sealed at both ends and dialysed against 45ml of 0.15 M NaCl in a 50 ml tube overnight. Using the diffusion model described, the optimum temperature for maximum glucose diffusion was established at 37 °C. Gentle shaking was used as well in a shaking incubator to simulate the effect of intestinal contractions on intestinal glucose absorption.[46] Thiebart-Fassy and Hervagault [47] reported that more vigorous stirring would have led to a decrease in the unstirred layer thickness, that is, to a decrease in the contribution of diffusional hindrance (whenever dietary fibres were included). The end point of glucose diffusion equilibrium (glucose diffusion into the external solution) was found by measuring the external solution glucose content in dialysate at 0, 3, 6, 18 and 24h time intervals. Glucose concentrations were measured in duplicates per time point-sample. The assay was internally controlled using 5 mM glucose solutions prepared alongside the experimental glucose samples.

To imitate the viscosity-based diffusion hindrance of gel-forming dietary fibres, and hence, their postprandial glucose lowering efficacies *in vitro*, guar gum 10, 25 and 50 mg/ml was used as a positive control, and 10, 25 and 50 mg/ml of plant AEs in 0.22 M glucoses in triplicates were dialysed against 0.15 M NaCl overnight at 37 °C with gentle shaking and a parallel plant-free (negative) control was included.[48]

Phytochemical screening: Ethanolic extracts (10%) of each of the three plants were subjected to TLC

examination for group determination of the secondary metabolites. Modified Dragendroff's reagent for alkaloids, ferric chloride reagent for phenolics, Naturstoff reagent for flavonoids, ethanolic KOH for coumarins and vanillin/sulphuric acid reagent for terpenoids were used. Solvent systems for the development of ready coated analytical TLC plates were selected according to Wagner and Bladt.[49]

Statistical analysis: The values are presented as mean ± S.E.M. (Standard Error of the Mean) of 3-4 independent experiments. Statistical differences between control and different treatment groups and A.U.Cs (incremental Area Under 24h-glucose Curve) were determined using Graphpad Prism one way analysis of variance (ANOVA) followed by Newman-Keuls post test whenever appropriate (version 3.02 for Windows; GraphPad Software, San Diego, CA, USA). A.U.Cs, also, were calculated by Graphpad Prism. Values were considered significantly different if $P<0.05$.

Conflict of interests: **The** authors declare that there are no conflicts of interests.

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Table 1 Phytochemical screening of plants extracts

Plant name	Alkaloids	Terpenoids	Flavonoids	Phenolics	Coumarins
Paronychia argentea	-	-	++	++	±
Rheum ribes	-	-	+	+++	-
Teucrium polium	-	++	++	++	+

Figure 1

(A)

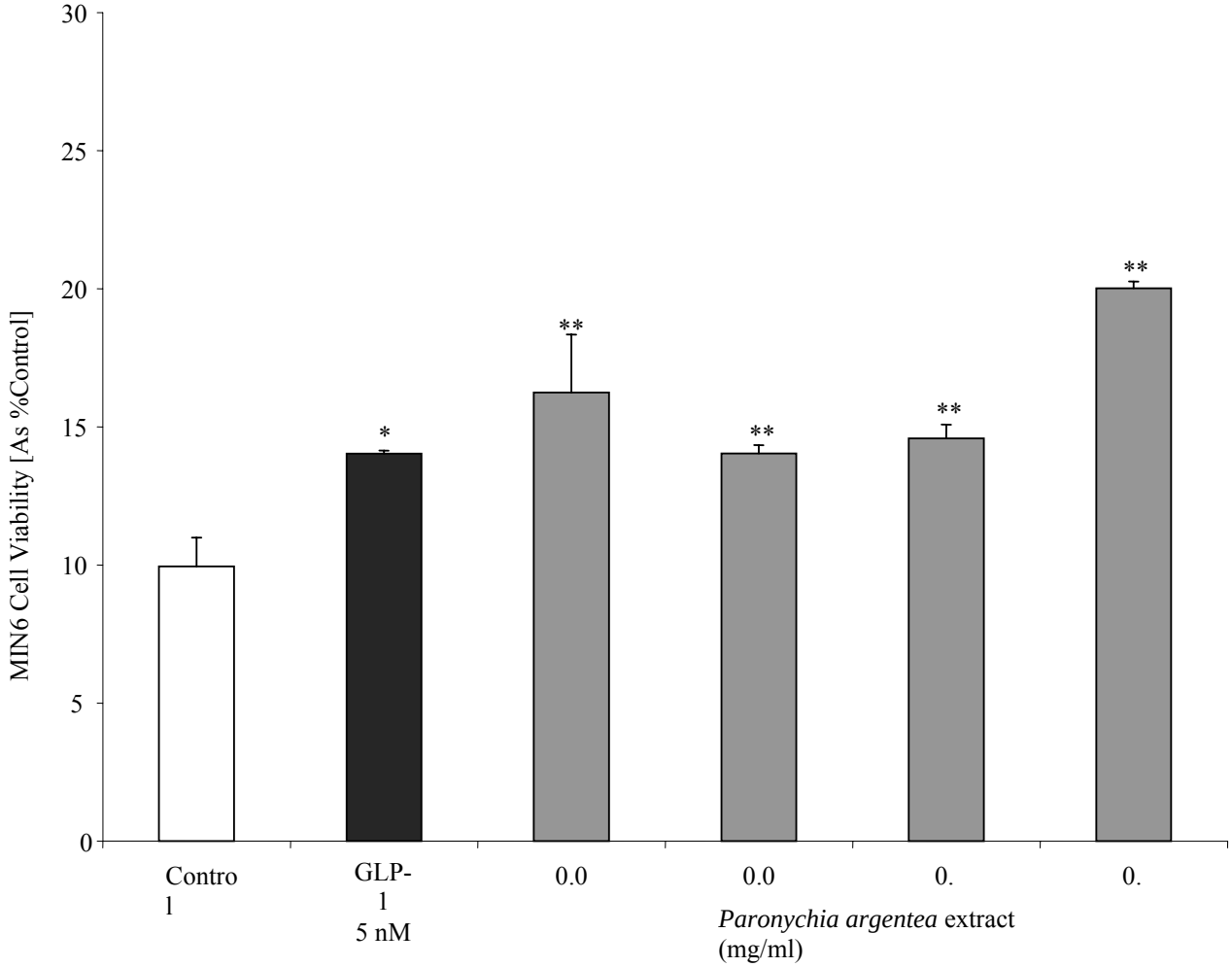
30
25
20
15
10
5
0
MIN6 Cell Viability [As %Control]
*
**
**
**
**
Contro
l
GLP-
1
5 nM
0.0
0.0
0.
0.
Paronychia argentea extract
(mg/ml)

Figure 1

(B)

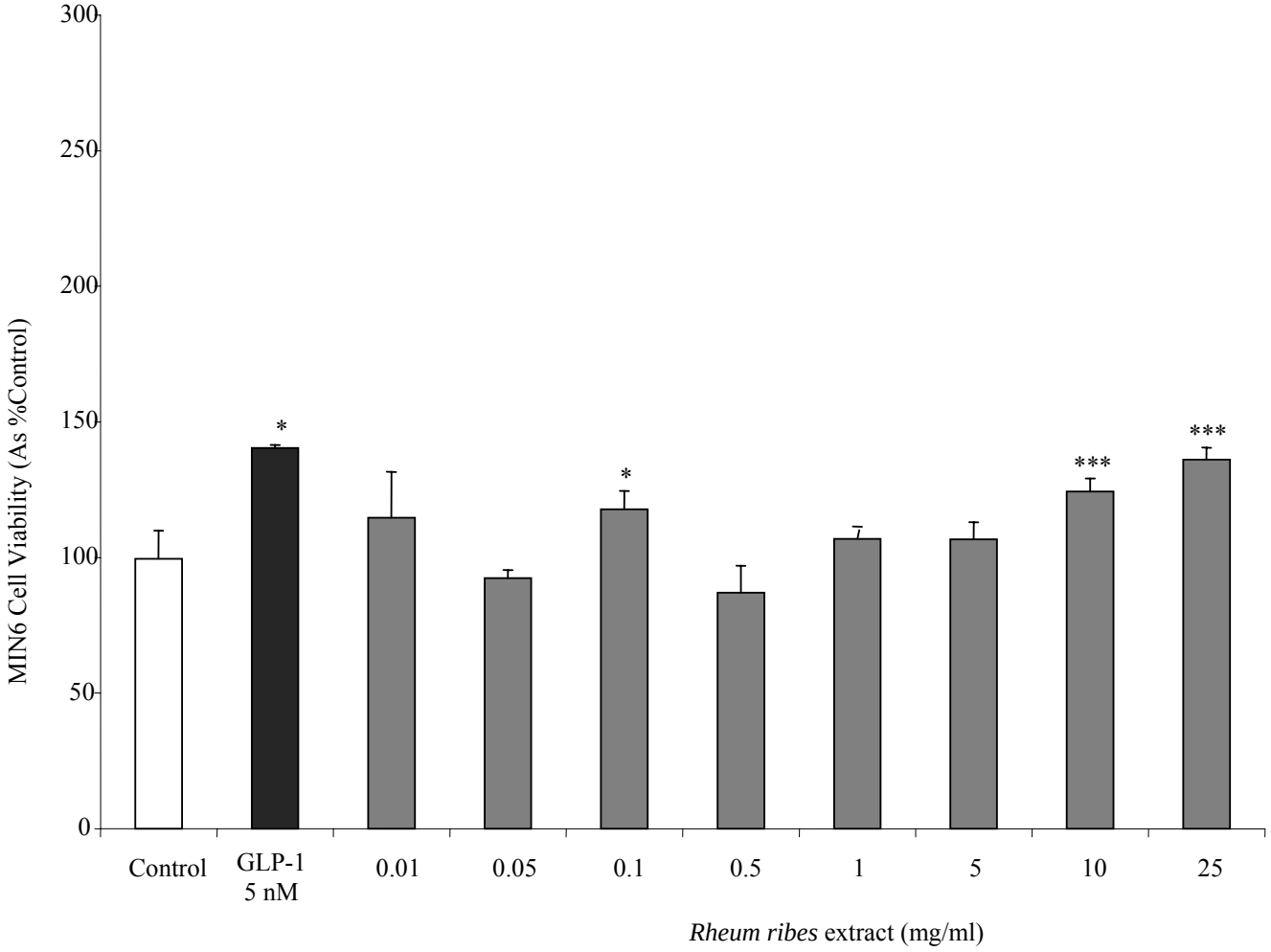
300
250
200
150
100
50
0
MIN6 Cell Viability (As %Control)
*
*
***
***
Control
GLP-1 5 nM
0.01
0.05
0.1
0.5
1
5
10
25
Rheum ribes extract (mg/ml)

Figure 1

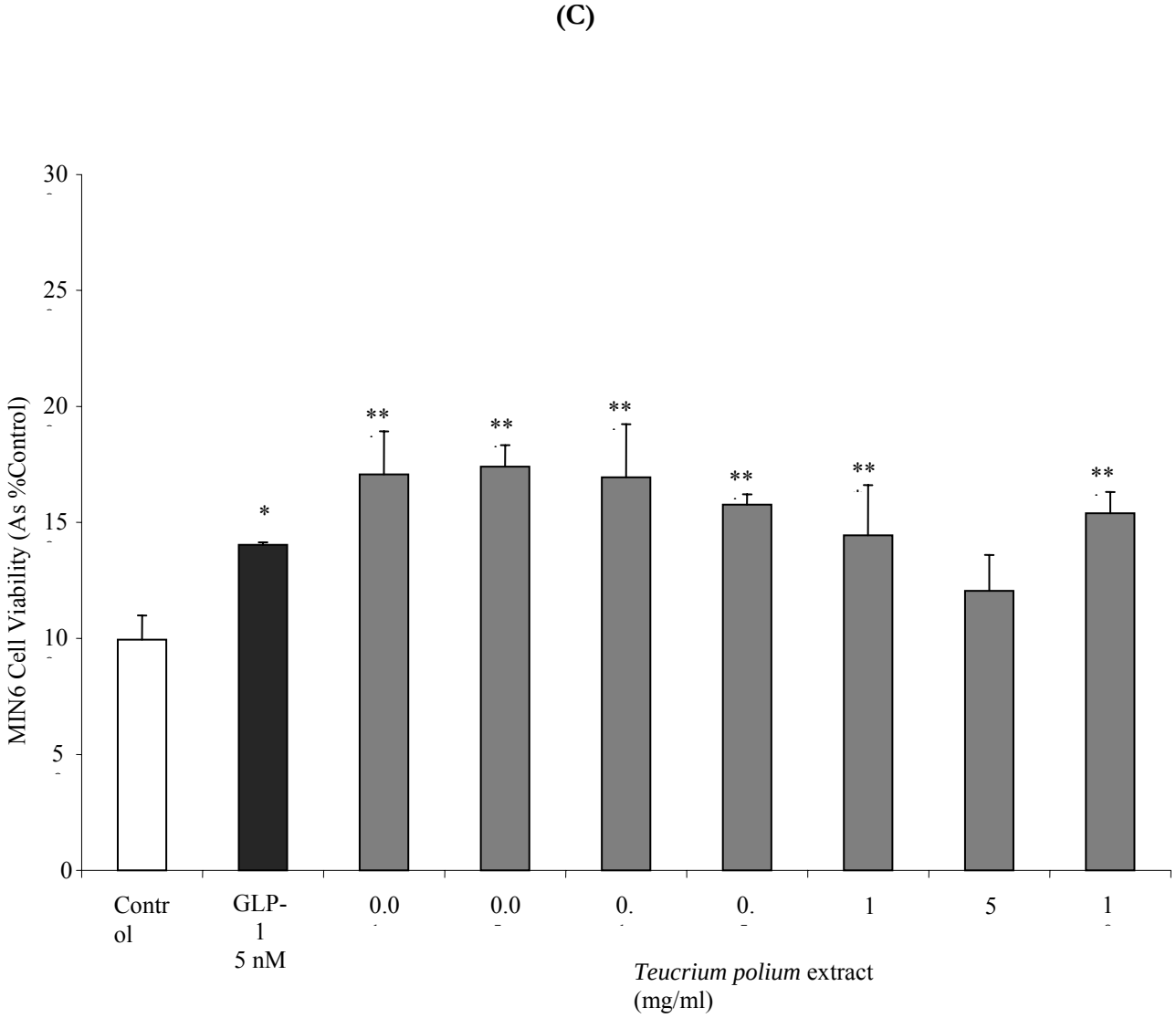

Figure 1: Modulatory effects of plants AE (0.01-25 mg/ml) on the proliferation of pancreatic β-cells MIN6 in 48h post seeding as measured by cell proliferation kit MTT. (A) *Paronychia argentea*. (B) *Rheum ribes*. (C) *Teucrium polium.* Each bar indicates the mean ± S.E.M of quadruplicate experiments. *P<0.05 and ***P<0.001 vs. untreated (spontaneous) control incubations.

Figure 2

(A)

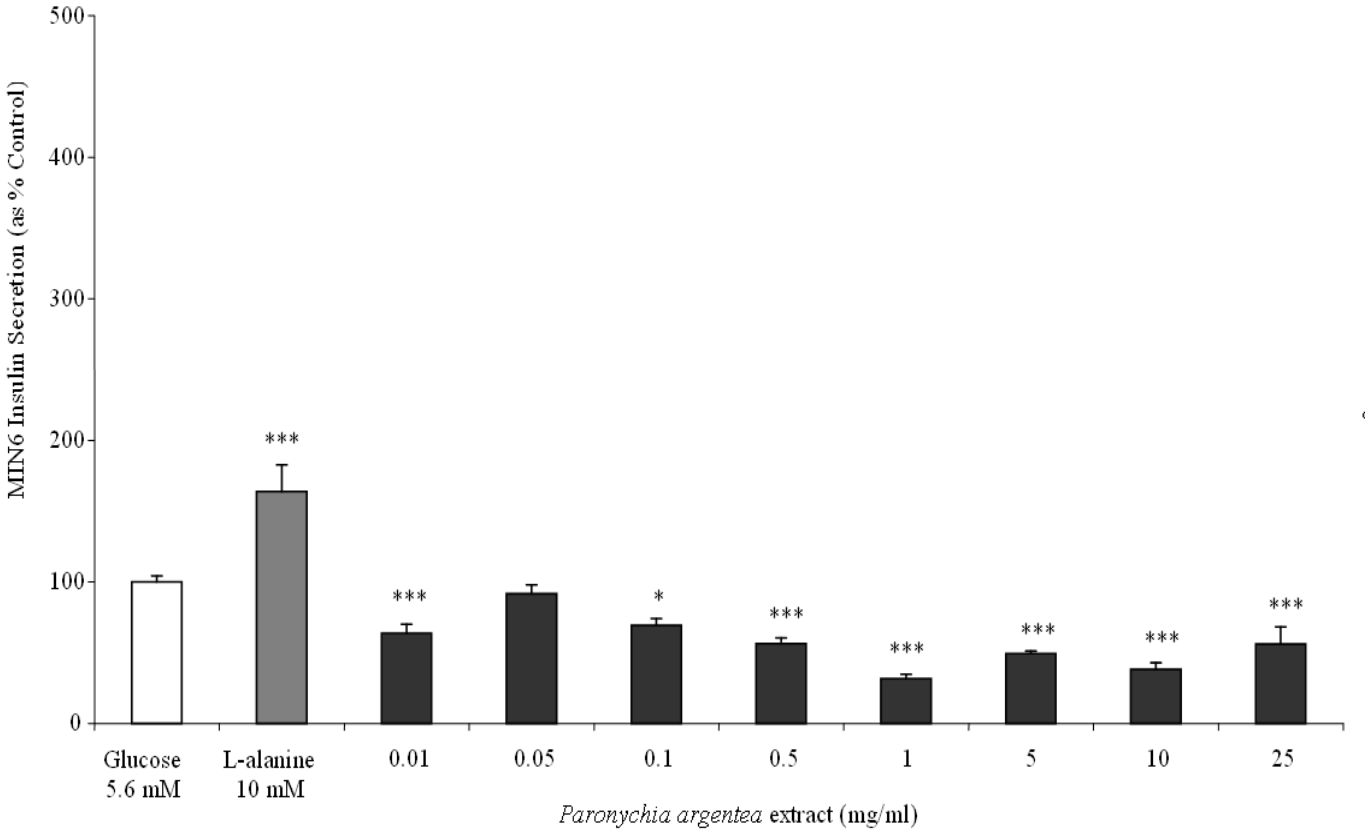
MIN6 Insulin Secretion (as % Control)
500
400
300
200
100
0
***
***
*
***
***
***
***
***
Glucose 5.6 mM
L-alanine 10 mM
0.01
0.05
0.1
0.5
1
5
10
25
Paronychia argentea extract (mg/ml)

Figure 2

(B)

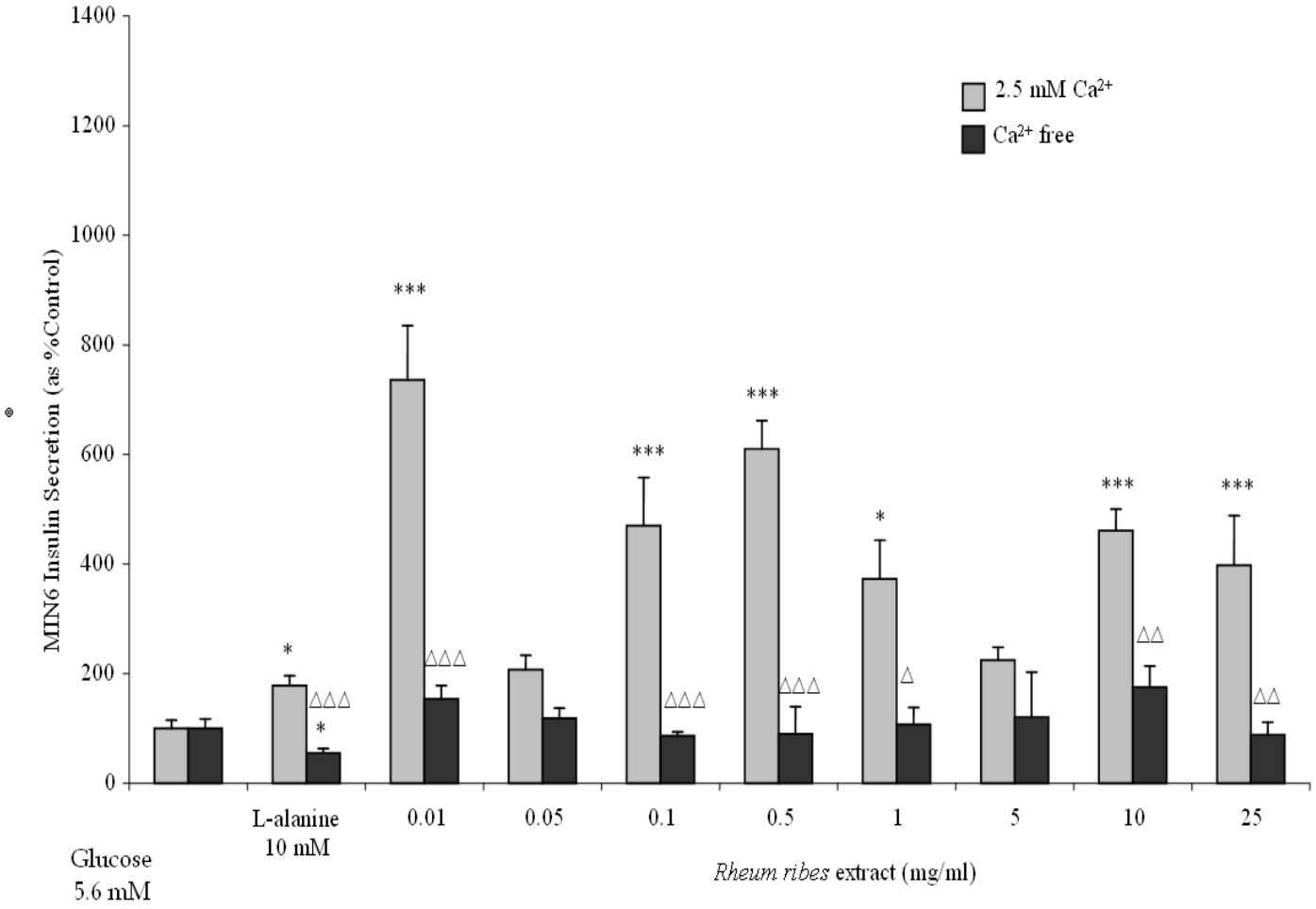
MIN6 Insulin Secretion (as %Control)
1400
1200
1000
800
600
400
200
0
2.5 mM Ca2+
Ca2+ free
L-alanine 10 mM
0.01
0.05
0.1
0.5
1
5
10
25
Glucose 5.6 mM
Rheum ribes extract (mg/ml)

Figure 2

(C)

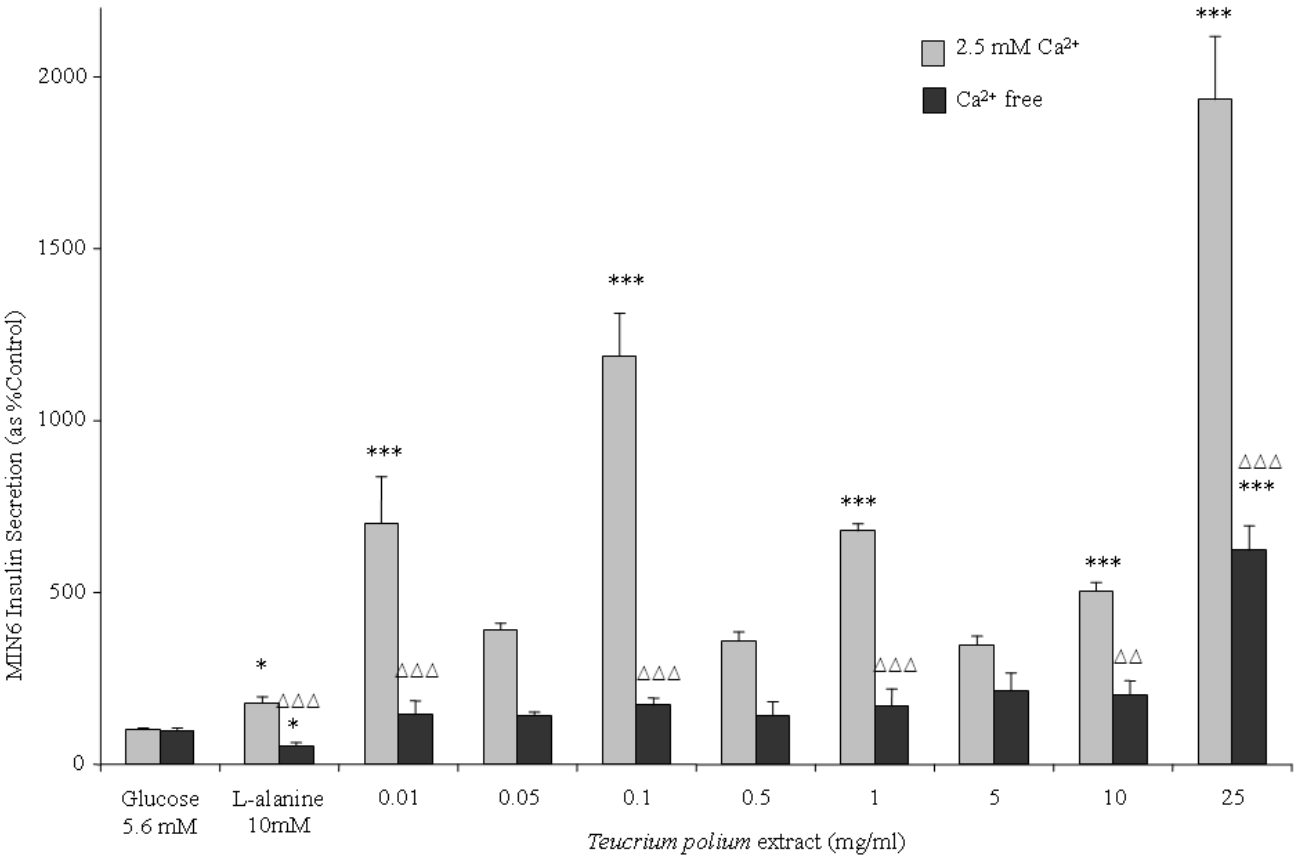

Figure 2: Modulatory effects of plants AE (0.01-25 mg/ml) on function of pancreatic β-cells MIN6. Such augmentation of GSIS following acute 1h - treatments was evaluated by rat insulin ELISA. (A) *Paronychia argentea*. (B) *Rheum ribes*. (C) *Teucrium polium.* Each bar indicates the mean ± S.E.M of quadruplicate determinations. Plant AE treatment wells were co-incubated in corresponding 5.6 mM glucose. *P<0.05 and ***P<0.001 vs. respective 5.6mM glucose (negative) control wells, while $^{\Delta}$P<0.05, $^{\Delta\Delta}$P<0.01 and $^{\Delta\Delta\Delta}$P<0.001 vs. respective treatment conditions in the presence of 2.5 mM Ca^{2+}

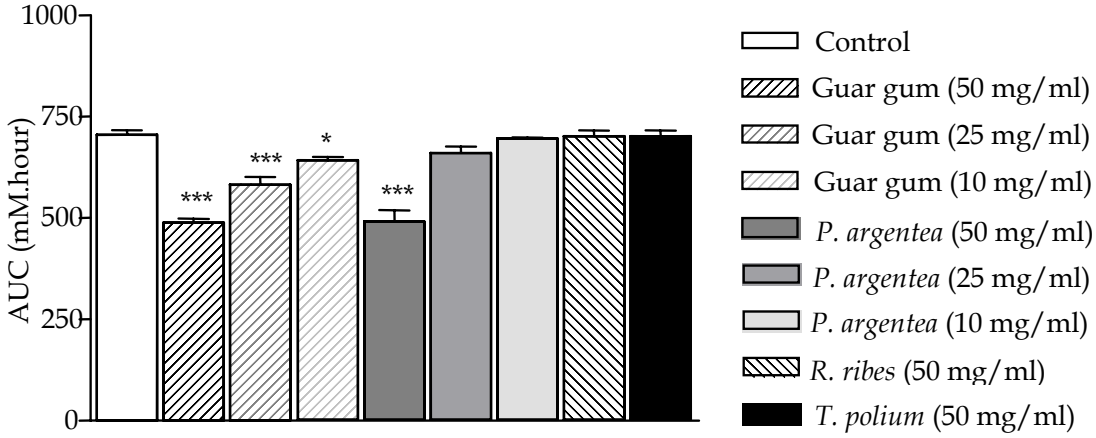

Figure 3: *Paronychia argentea* (10, 25 and 50 mg/ml) and *Rheum ribes* and *Teucrium polium* AE (50 mg/ml each) modulate the incremental AUC of 24h glucose movement *in vitro*. Each bar indicates the mean ± S.E.M of triplicate independent experiments. ***P<0.001 vs. overnight negative control (plant-free) incubations.

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دراسة تأثير مستخلصات Paronychia argentea, Rheum ribes و Teucrium poluim على افراز الانسولين والتضاعف الخلوي البنكرياسي وامتصاص الجلوكوز الخارج بنكرياسي.

فيوليت كسابري، رنا ابو الذهب، فاطمة عفيفي، رندة نفاع، لارا مجدلاوي، هزار شواش.

كلية الصيدلة، كلية الطب، الجامعة الأردنية.

ملخص

يتحقق تصميم الدراسة الحالي من تأثير المستخلصات الخام لكل من *Paronychia argentea, Rheum ribes,* *Teucrium polium* والتي تستخدم تقليديا في علاج مرض السكري في الاردن على تضاعف خلايا MIN6 البنكرياسية وافرازها للأنسولين وامتصاص الجلوكوز خارج البنكرياس. في ترتيب تصاعدي ان تراكيز كل من *R.ribes,* *T,polium and P.argentea* حفزت توسع طبقات MIN6 ($P<0.001$) بالتالي هي تزيد بذلك عن قدرة GLP−1 (5 nM) على تحفيز التكاثر البنكرياسي. ومثل قدرة L-alanine (10 mM) على تحفيز افراز الانسولين بدون اي تأثير سمي فإن كلا من جرعات *T.polium* و*R.ribes* قد حفزت من افراز المزيد من الانسولين ولكن *P.argentea* كانت غير فاعلة. ان نضب Ca^{+2} قد الغى تأثير النباتات المفرزة للأنسولين. بالمقارنة مع اعاقة Guar gum لانتشار الجلوكوز فان *P.argentea* حالت دون حركة الجلوكوز عبر غشاء nitrocellulose ($p<0.001$) ولكن *T.polium* و *R.ribes* كانت غير فاعلة بالمقارنة.

ان هذه التقييمات المخبرية قد كشفت أن النباتات الثلاث زادت من توسع الخلايا البنكرياسية وأن *P.aragentea* منعت امتصاص السكويات بينما حفزت كل من *T.polium* و*R.ribes* من افراز الانسولين. هذه الاجراءات تعتمد على استيعابهم السليم في الجسم. وقد تتأتى التوجيهات المستقبلية من تقييم اسخدامها كعناصر غذائية بصفات فاعلة ومصادر للعلاج الدوائي لمرضى السكري.

الكلمات الدالة: افراز الأنسولين، التضاعف الخلوي البنكرياسي، امتصاص الجلوكوز.

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