Punica granatum Peel Extract Stimulates Insulin Secretion from Clonal Pancreatic BRIN-BD11 β-cells and Improves Glucose Homeostasis in High-Fat-Fed Diet-Induced Obese Mice

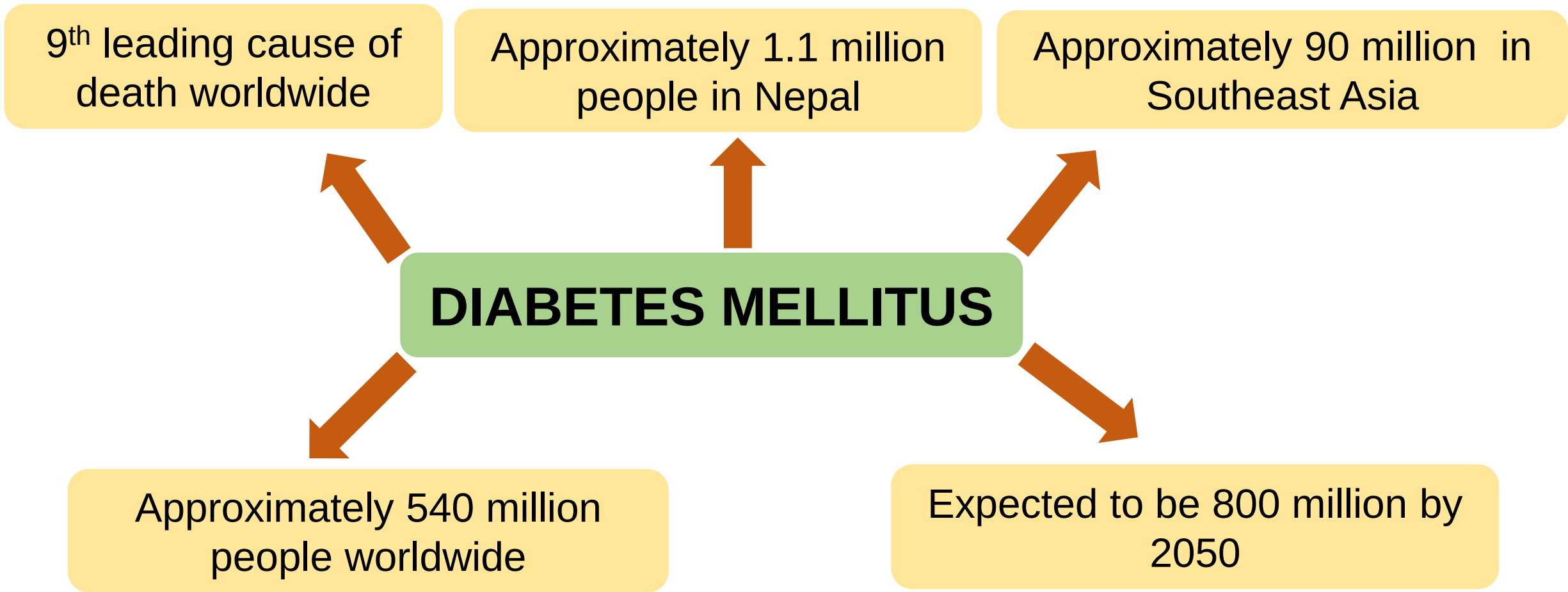
Prawej Ansari[1,2,3*], Nushrat J. Ansari[1], J.M.A. Hannan[2], Yasser H.A. Abdel-Wahab[3], Peter R. Flatt[3]

[1]Department of Pharmacology* and Radiology, National Medical College, Birgunj, Nepal
[2]Department of Pharmacy, Independent University, Bangladesh (IUB), Dhaka, Bangladesh
[3]Centre for Diabetes, School of Biomedical Sciences, University of Ulster, Coleraine, UK

BACKGROUND

- **Diabetes mellitus:** A chronic metabolic disorder characterized by defective insulin action, secretion or both
- Long term hyperglycaemia leads to various complications including retinopathy, neuropathy, nephropathy and cardiovascular diseases
- There are major two types of diabetes – type 1, type 2
- **Type 1:** Due to autoimmune destruction of β-cells
- **Type 2:** Due to Obesity and Insulin resistance

CURRENT STATUS OF DIABETES



PUNICA GRANATUM (Peel)

- Pomegranate peel, rich in potent polyphenolic compounds.

- It has a long history of traditional use for various ailments:
  - -Diarrhea
  - -Dysentery,
  - -Oral health issues like gum problems, and wound healing

- Modern research highlights pomegranate peel potential in
  - -Improving insulin sensitivity
  - -Reducing blood glucose levels
  - -Potent antioxidant & anti-inflammatory effects

- Research indicates that **Anthocyanins** may improve insulin sensitivity, blood sugar levels, & enhance glucose uptake.

AIMS/OBJECTIVES

This project aimed to investigate the potential of Ethanol extracts of *Punica granatum* peel (EEPG) to improve glucose homeostasis through:

In vitro:

- Determining the dose-dependent insulinotropic effects of EEPG on BRIN-BD11 β-cells.
- Elucidating the insulin secretory pathways modulated by EEPG in BRIN-BD11 β-cells.
- Evaluating the effect of EEPG on β-cell proliferation.

In vivo:

- Assessing the impact of EEPG on oral glucose tolerance in high-fat diet-induced obese mice.
- Analyzing the effects of EEPG on FBG, body weight, food and fluid intake.
- Evaluating the effects of EEPG on Gut motility.

METHODOLOGY

Acute insulin releasing studies on BRIN-BD11 cells for insulin secretory action

Measurement of β-cell proliferation in BRIN-BD11 cells

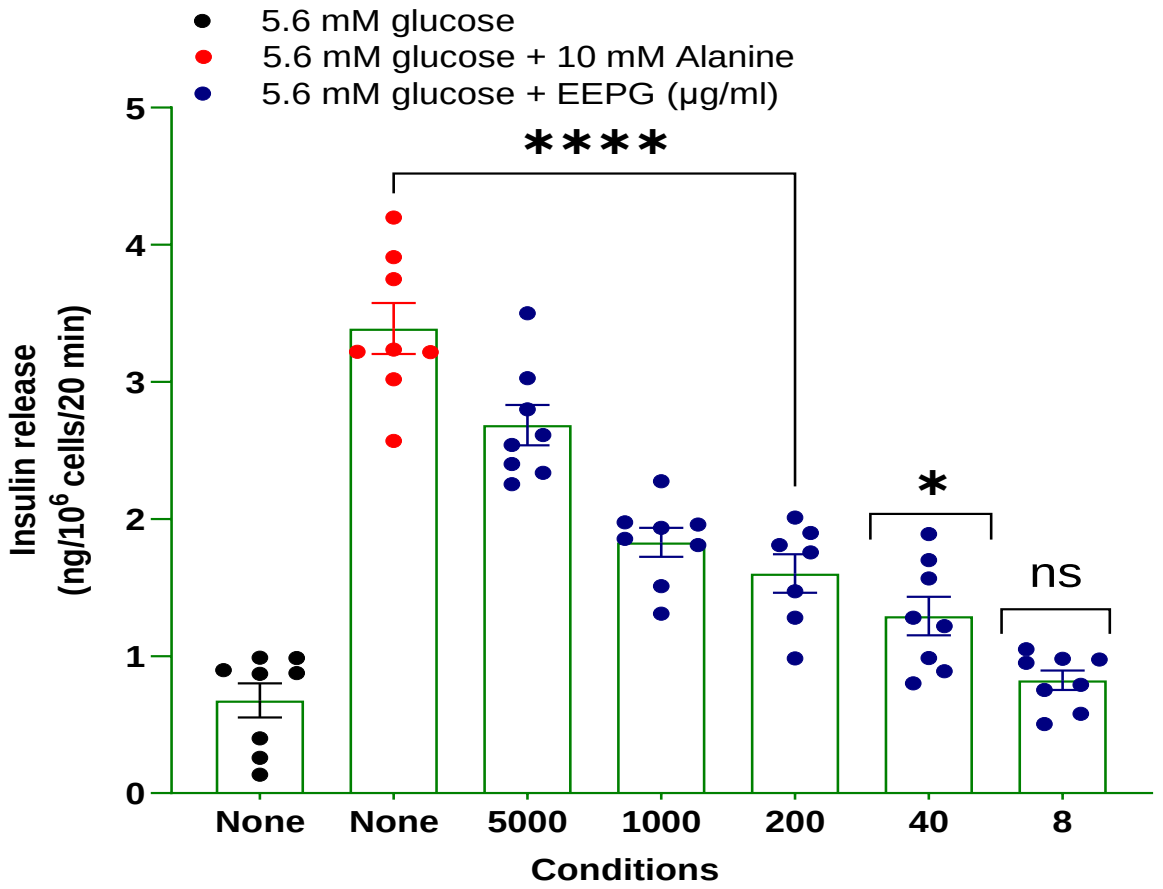
Acute oral glucose tolerance test and plasma insulin

Chronic studies includes FBG, Body weight, Food and Fluid intake

Phytochemical screening

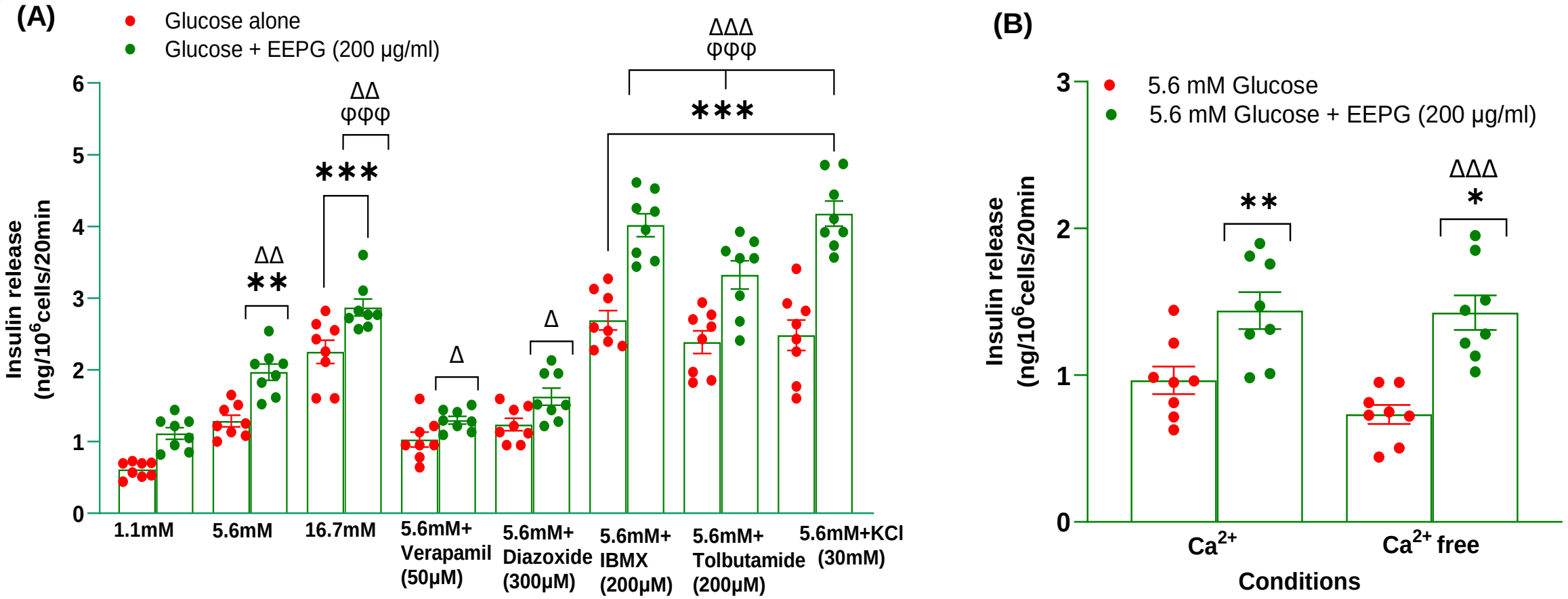
RESULTS

Fig 1: Dose-dependent effects of various concentration of ethanol extract of *Punica granatum peel* at 5.6mM glucose on insulin release from BRIN-BD11 cells



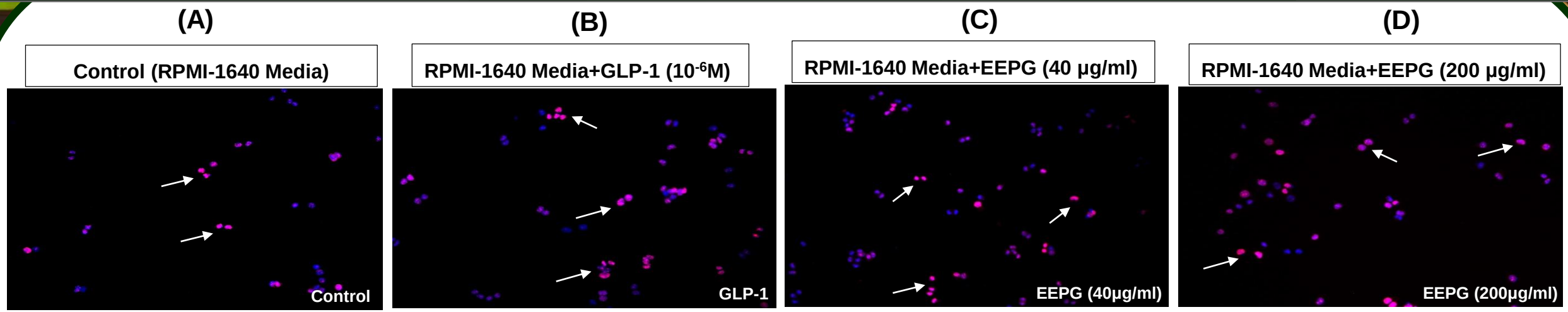
Insulin release was measured after 20-min incubation with a range of concentrations (5000 to 8 µg/ml) of ethanol extract of *Punica granatum peel* at 5.6 mM glucose, whereas Alanine (10 mM) were used as a reference control. Values are Mean±SEM with n = 8. *P<0.05, **P<0.01 and ***P<0.001 compared to 5.6 mM glucose.

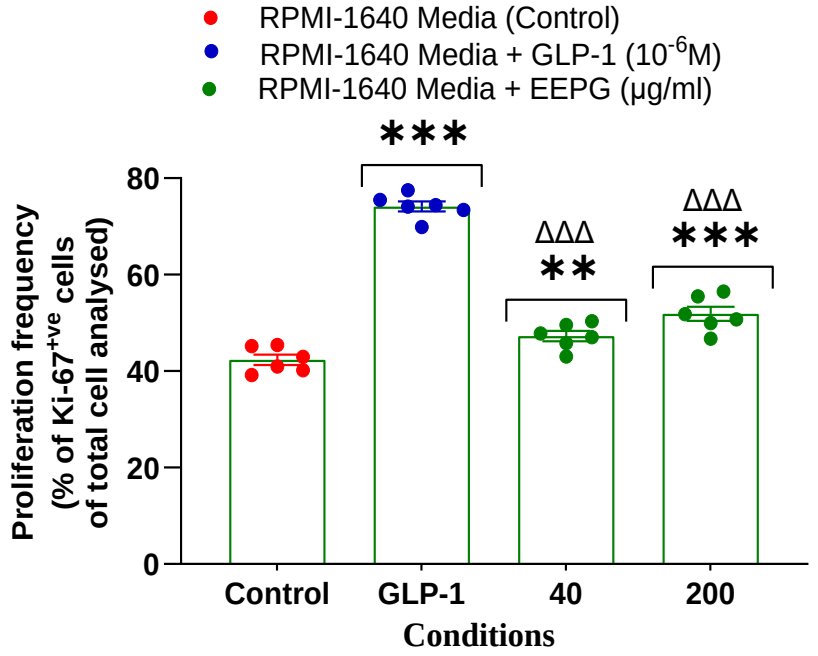
Fig 2: Effects of ethanol extract of *Punica granatum* peel on insulin release from BRIN-BD11 cells (A) in the presence of insulin inhibitors/modulators and (B) in the absence of extracellular calcium



Insulin release was measured after 20-min incubation with EEPG in the presence and absence of insulin modulators/inhibitors at 5.6 mM and 16.7 mM glucose.Values are Mean±SEM with n = 8. *P<0.05, **P<0.01 and ***P<0.001 compared to 5.6 mM glucose alone (in the presence of extracellular calcium). $^{\Delta}$P<0.01, $^{\Delta\Delta}$P<0.01 and $^{\Delta\Delta\Delta}$P<0.001 compared to 5.6 mM glucose in the presence of the EEPG. $^{\Phi\Phi\Phi}$P<0.001 compared to respective incubation in the absence of the plant extract.

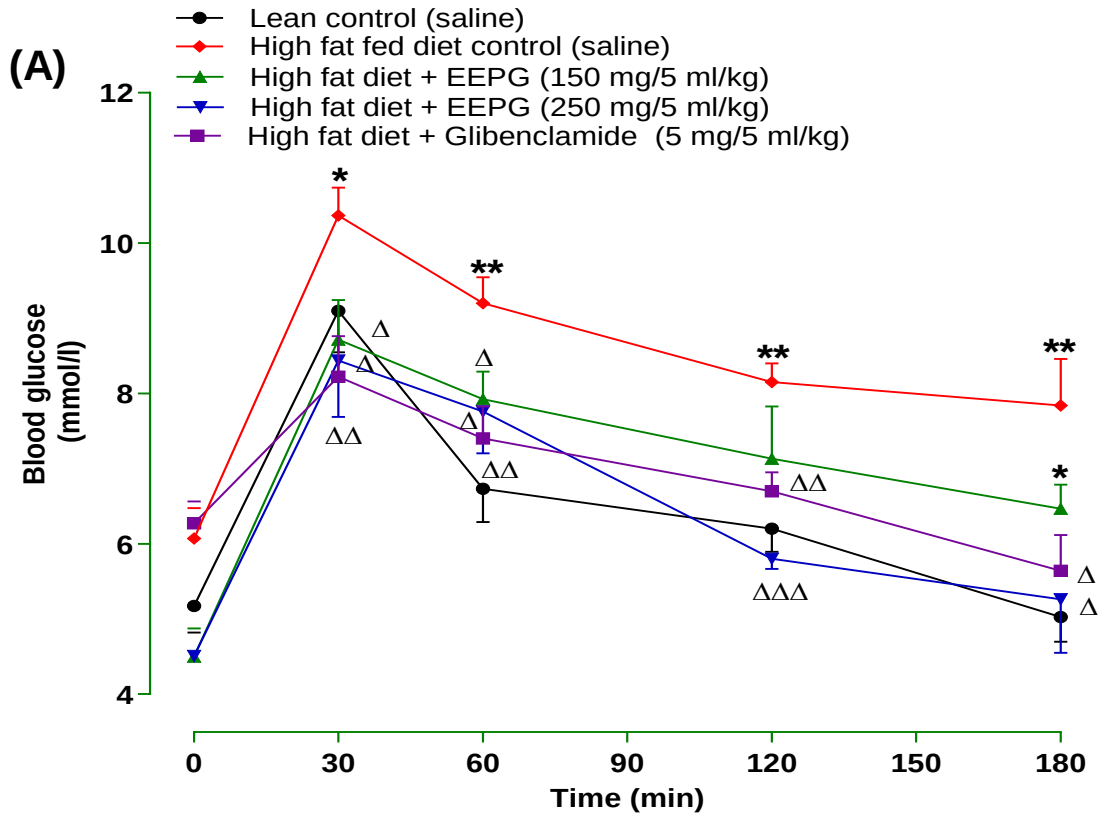
Fig 3: Effects of Ethanol Extract of *Punica granatum* peel on β-cell proliferation in BRIN BD11 cells

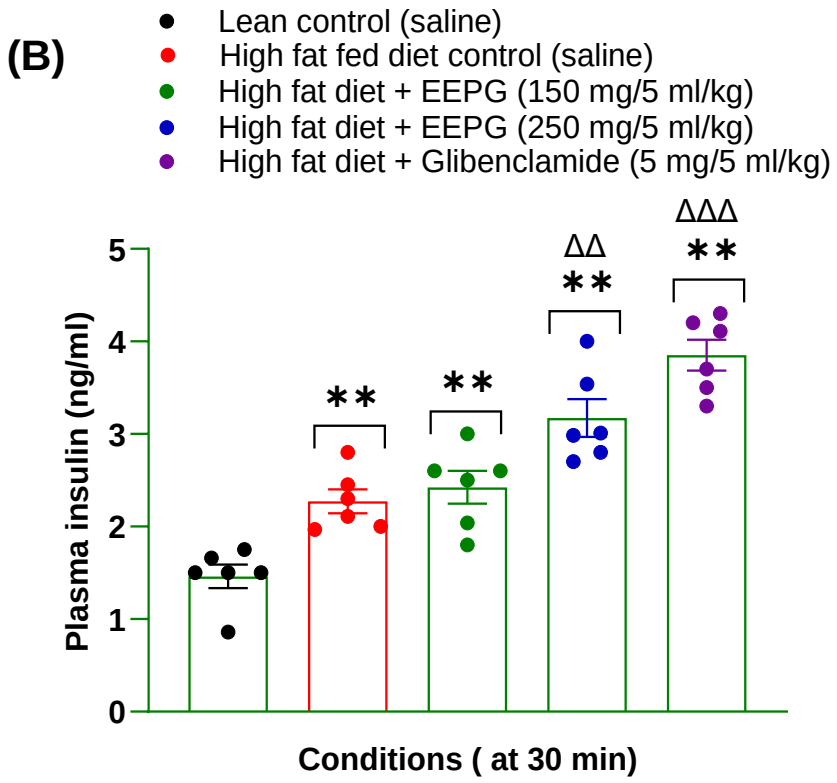




Effects of EEPG on β-cell proliferation in BRIN-BD11 cells compared with 1 µM GLP-1. Values are Mean±SEM with n=6. **P<0.01, ***P<0.001 compared with incubation in culture medium alone, $^{\Delta\Delta\Delta}$P<0.001 compared to GLP-1 treated cells.

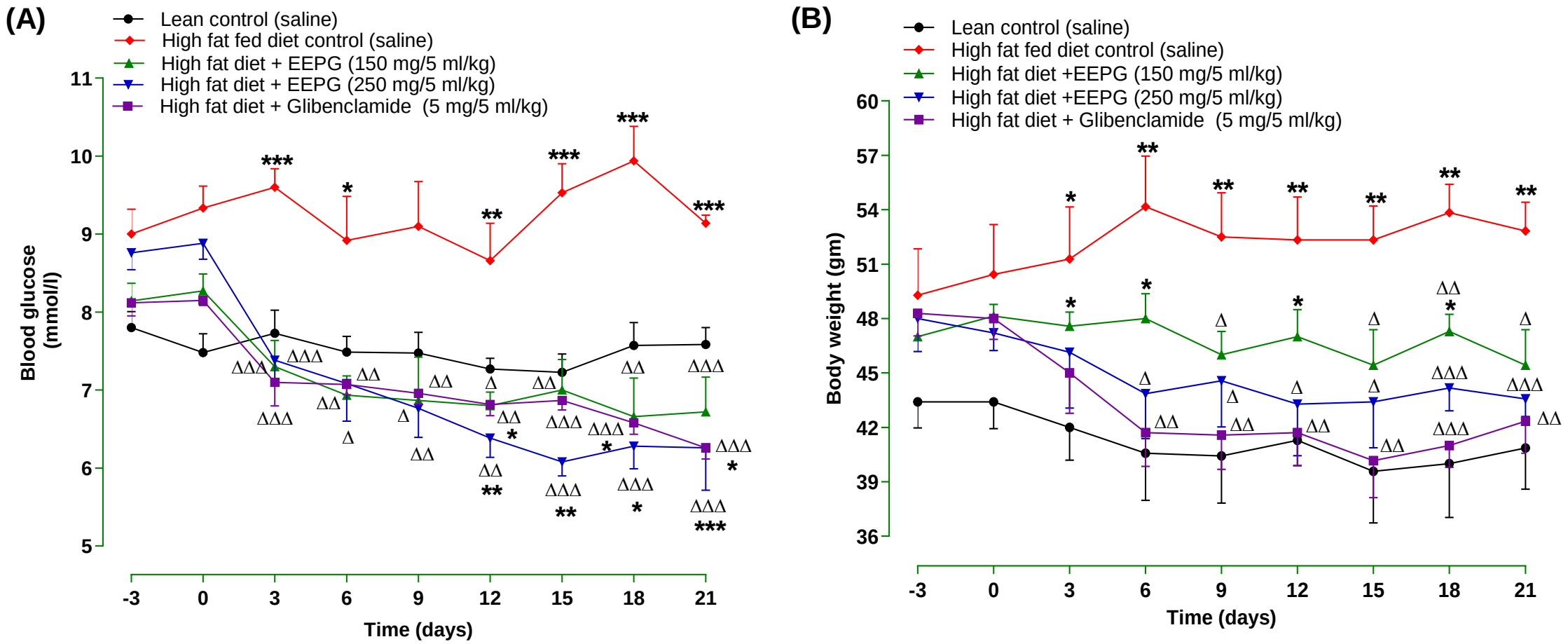
Fig 4: Effects of Ethanol Extract of *Punica granatum* peel on (A) oral glucose tolerance and (B) plasma insulin





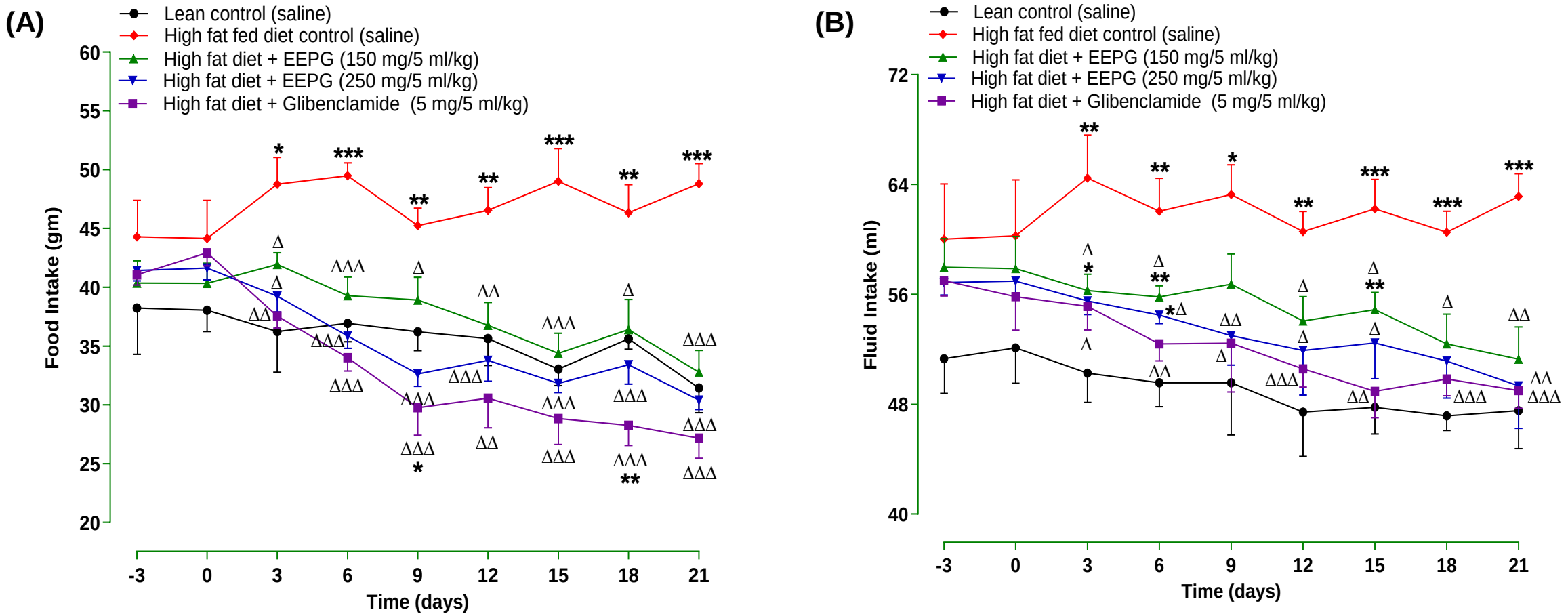
Oral glucose tolerance and plasma insulin were evaluated before and after oral administration of glucose alone (18 mmol/kg body weight, control) or with EEPG (150 and 250 mg/5 ml/kg body weight). Values are Mean±SEM with n = 6, *P<0.05, **P<0.01 and ***P<0.001, compared to lean control and $^{\Delta}$P<0.01, $^{\Delta\Delta}$P<0.01 and $^{\Delta\Delta\Delta}$P<0.001 compared to high fat fed diet control.

Fig 5: Effects of Ethanol Extract of *Punica granatum* peel on (A) Fasting blood glucose and (B) Body weight



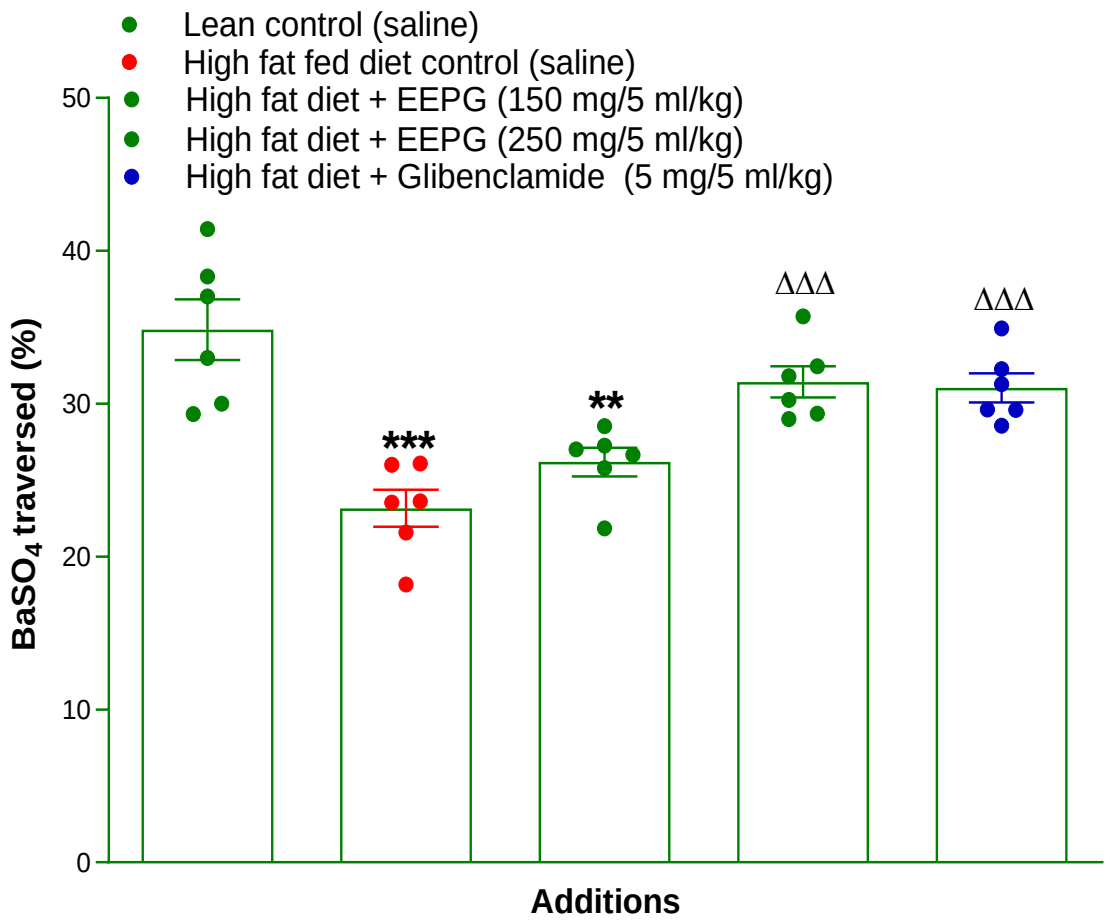
Fasting blood glucose and body weight were measured every three days over a 21-day study period, during which EEPG (150 and 250 mg/5 ml/kg body weight) was administered via oral gavage twice daily. Values are Mean±SEM with n = 6, *P<0.05, **P<0.01 and ***P<0.001, compared to lean control and $^{\Delta}$P<0.01, $^{\Delta\Delta}$P<0.01 and $^{\Delta\Delta\Delta}$P<0.001 compared to high fat fed diet control.

Fig 6: Effects of Ethanol Extract of *Punica granatum* peel on (A) Food and (B) Fluid intake



Food and fluid intake were measured every three days over a 21-day study period, during which EEPG (150 and 250 mg/5ml/kg body weight) was administered via oral gavage twice daily. Values are Mean±SEM with n = 6, *P<0.05, **P<0.01 and ***P<0.001, compared to lean control and $^{\Delta}$P<0.01, $^{\Delta\Delta}$P<0.01 and $^{\Delta\Delta\Delta}$P<0.001 compared to high fat fed diet control.

Fig 7: Effects of Ethanol Extract of *Punica granatum* peel on Gut Motility



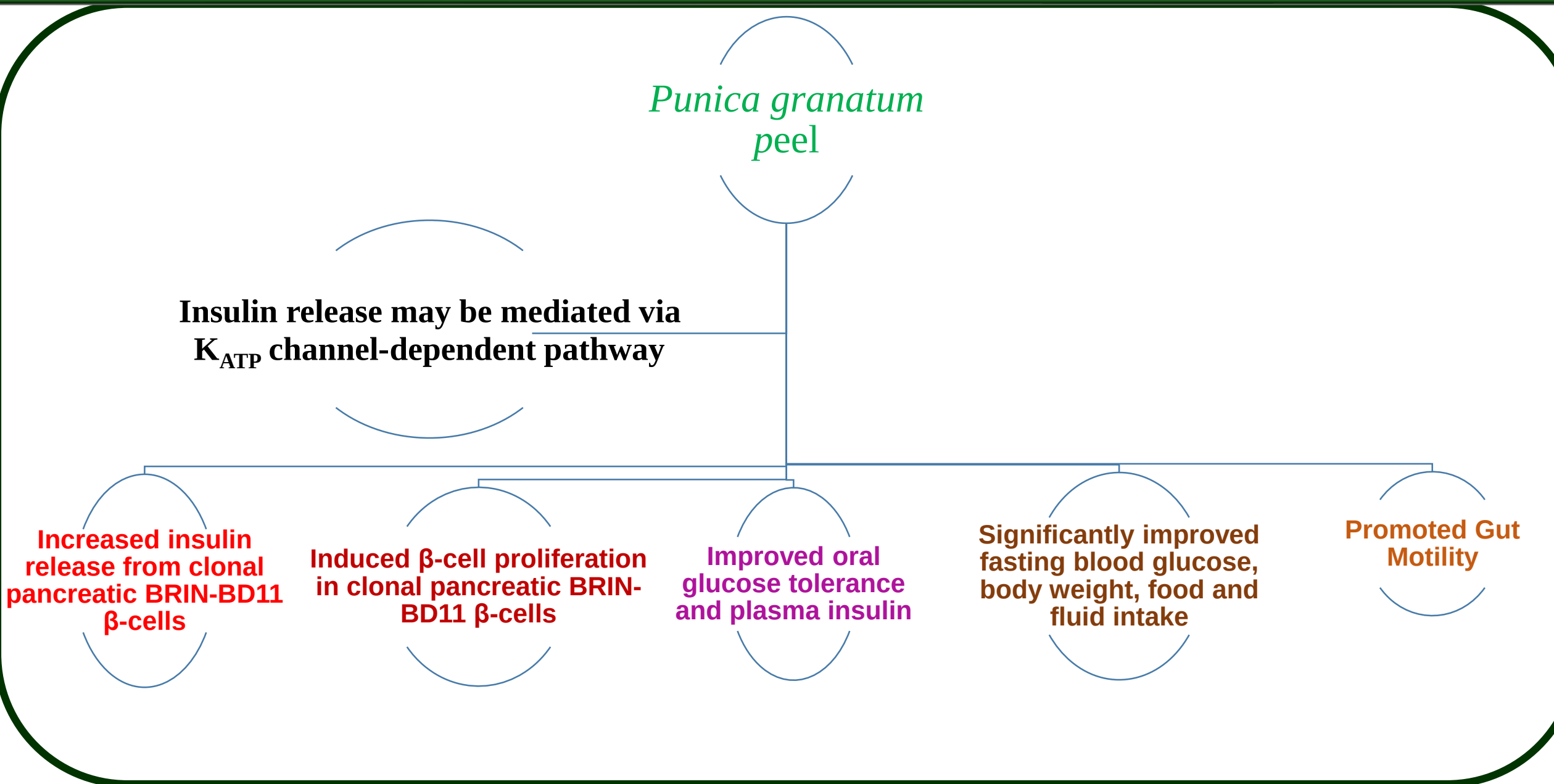
Following 21 days of twice-daily oral gavage with EEPG (150 and 250 mg/5ml/kg), the animals were sacrificed, and gut motility was measured. Values are Mean±SEM with n = 6, **P<0.01 and ***P<0.001, compared to lean control and $^{\Delta\Delta\Delta}$P<0.001 compared to high fat fed diet control.

PHYTOCHEMICAL SCREENING

Phytochemical Group	Specific Phytochemicals Present in EEPG Peel
Polyphenols	Ellagic acid, Gallic acid, Quercetin, Catechins, Anthocyanins, p-coumaric acid
Tannins	----------
Terpenoids	----------
Steroids	----------
Alkaloids	----------
Saponins	----------

Recent Findings suggests that anthocyanins in EEPG are likely responsible for its beneficial effects on blood sugar, insulin secretion, body weight, and beta-cell health, further research is required to confirm this.

CONCLUSION


Punica granatum peel
Insulin release may be mediated via K_{ATP} channel-dependent pathway
Increased insulin release from clonal pancreatic BRIN-BD11 β-cells
Induced β-cell proliferation in clonal pancreatic BRIN-BD11 β-cells
Improved oral glucose tolerance and plasma insulin
Significantly improved fasting blood glucose, body weight, food and fluid intake
Promoted Gut Motility

FUTURE DIRECTION

- Need to Identify the bioactive compounds in EEPG peel, and elucidate their mechanisms of action in improving beta-cell function and lowering blood sugar.

- Need of clinical trials to confirm EEPG's safety and effectiveness as a dietary supplement for diabetes.

- Finally, Need to explore if EEPG's components can be developed into novel oral diabetes medications.

ACKNOWLEDGMENTS

I would like to thank our collaborators, **Prof. Peter R. Flatt**, **Dr. Yasser Abdel-Wahab**, and **J.M.A. Hannan**, for their insightful contributions to the completion of this project.

Prof. Peter R. Flatt

Dr. Yasser Abdel-Wahab

Prof. J.M.A. Hannan

BIOGRAPHY

Dr. Prawej Ansari holds a **PhD** in **Pharmacology** from the University of Ulster, UK. He began his career as a **Scientist** at Randox Laboratories Ltd., Antrim, UK. Subsequently, he served as an **Assistant Professor** at Independent University, Bangladesh. Dr. Ansari recently served a **Visiting Scientist** at the University of Alabama, Birmingham, USA, and is currently an **Assistant Professor** at National Medical College, Nepal, and **Editor-in-Chief** of Medphoenix, Journal of National Medical College. His research in **diabetes and endocrinology** is evidenced by **57 peer-reviewed articles**, **12+ conference proceedings**, **1000+ citations**, and editorial work for journals like Metabolites, Frontiers in Pharmacology, and International Journal of Molecular Sciences. He has reviewed **200+ articles** and presented his research findings at **12+ international conferences**.

THANK YOU