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Insulin secretory actions of polyphenols of *Momordica charantia* regulate glucose homeostasis in alloxan-induced type 2 diabetic rats

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Abstract

Objective: *Momordica charantia*, commonly known as bitter gourd, is traditionally used as remedies for various diseases including diabetes. The main objective of this study is to investigate the *in vitro* and *in vivo* insulinotropic and antidiabetic effects of an 80% ethanolic extract of *M. charantia* (EEMC) fruit, as well as the underlying molecular mechanism involved and preliminary phytochemical screening.

Methods: The insulin secretion was measured using clonal pancreatic BRIN-BD11 β-cells and isolated mouse islets. The ability of EEMC to inhibit carbohydrate digestive enzymes and glucose absorption and to scavenge free radicals was assessed via starch digestion, glucose diffusion, and 2,2-diphenyl-1-picrylhydrazyl assay methods. The effects of EEMC on a variety of metabolic parameters were evaluated in alloxan-induced type 2 diabetic rats, including lipid profile. Finally, a preliminary phytochemical screening was conducted to identify the active phytoconstituents.

Key findings: EEMC increased insulin release through the K_{ATP}-dependent/cyclic adenosine monophosphate pathway, which depolarizes the β-cell membrane and elevates intracellular calcium. It also inhibited glucose absorption and free radicals, suggesting its potential to delay gastric emptying, attenuate oxidative stress, and reduce inflammatory cytokines. *In vivo* studies showed that EEMC improves oral glucose tolerance, food intake, fasting blood glucose, plasma insulin, and lipids and promotes intestinal motility. The active phytoconstituents in EEMC, such as flavonoids, alkaloids, tannins, saponins, steroids, and glycosides, are likely responsible for these effects.

Conclusion: The antihyperglycemic properties of EEMC indicate that it might be a promising candidate for diabetes management. However, additional study into the application of *M. charantia* in type 2 diabetes is essential.

Keywords: insulin; glucose; type 2 diabetes mellitus; DPPH; lipids; *Momordica charantia*

Introduction

Diabetes mellitus is a deleterious metabolic disorder characterized by a high concentration of blood glucose levels, caused by a lack of or resistance to the hormone insulin [1]. According to the International Diabetes Federation 2021 data, approximately half a million people globally are suffering from diabetes, and this number is estimated to increase to 783 million by the year 2045 [2]. People who are diagnosed with diabetes primarily fall under one of the two major categories—type 1 diabetes mellitus and type 2 diabetes mellitus (T2DM). Significant reduction in insulin secretion due to almost absolute destruction of pancreatic β-cells results in type 1 diabetes [3], while both insulin resistance and insulin secretion deficiency in pancreatic β-cells contribute to type 2 diabetes [4]. Obesity and physical inactivity have also been found as the primary risk factors for increasing prevalence of T2DM, making it the predominant form that accounts for nearly 90% of all cases [5, 6]. Long-term lack of insulin action on target tissues can lead to abnormalities in the metabolism of carbohydrates, lipids, and proteins. This condition exacerbates hyperglycemia and damages the blood vessels of brain, retina, heart, and kidney, ultimately contributing to severe macro- and microvascular complications such as cardiovascular diseases (CVDs), cerebral and peripheral vascular diseases, neuropathy, retinopathy, nephropathy, and even foot amputation [7–10].

The current interventions for treating and managing the disease include diet, weight reduction, and exercise together with the controlled use of single or combined oral antihyperglycemic agents such as metformin, sulfonylureas, thiazolidinediones, Dipeptidyl Peptidase-IV (DPP-IV) inhibitors, Sodium-Glucose Co-Transporter 2 (SGLT-2) inhibitors, α-glucosidase inhibitors, glucagon-like peptide 1 (GLP-1) mimetics, or insulin [9, 11, 12]. Although these drugs are effective in enhancing insulin secretion and decreasing insulin resistance, they are often costly, accompanied by multiple side effects, and inaccessible to the majority of the population mostly in underdeveloped countries [9, 13]. This necessitates

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the development of alternative therapies like herbal remedies and dietary supplements for global diabetes control and management.

The traditional use of plants has been practiced for thousands of years in treating various ailments as it holds the rich source of pharmacologically potent bioactive components with fewer adverse effects. More than 800 plant species have been reported to exhibit antidiabetic activity while several others are still being investigated [14]. These medicinal plants include numerous classes of phytochemicals such as flavonoids, alkaloids, terpenoids, saponins, tannins, phenolic, and glycosides that have demonstrated safe and effective insulinotropic and antidiabetic properties [15].

Momordica charantia, widely known as bitter gourd or bitter melon, belonging to the Cucurbitaceae family, is cultivated in tropical and subtropical regions worldwide [16]. It serves not only as a vegetable but also as a potent ethnomedicine with diverse medicinal properties, including antidiabetic, anti-inflammatory, and antibacterial effects [17]. Traditionally used for various ailments, its fruits are particularly effective in controlling hyperglycemia [18]. Studies suggest it repairs β-cells, enhances insulin levels and sensitivity, inhibits glucose absorption, and stimulates thyroid hormone synthesis [19]. Active compounds like vicine, charantin, and polypeptide-p mimic insulin and improve insulin sensitivity [20]. This study investigates the insulin-releasing, glucose-lowering, lipid-lowering, and antioxidant properties of *M. charantia* fruit using both *in vitro* and *in vivo* approaches to elucidate the underlying mechanisms of action.

Materials and methods

Collection and preparation of plant extract

Momordica charantia fruits were purchased from Sreebardi Upazila, Sherpur, Bangladesh. Its authenticity was confirmed by a botanical taxonomist and assigned herbarium number DACB 66954. The fruits were thoroughly washed and allowed to air-dried before being dried in an oven at 40°C, and pulverized in a grinding mill. The dry powder was immersed in 80% ethanol and shaken in an orbital shaker at 950 rpm for 48–72 h. The mixture was filtered using Whatman filter paper, and any excess solvent was vaporized using a rotary evaporator (Bibby RE-200, Sterilin Ltd., Atkinson, UK). The gummy residue was lyophilized, and then labeled and stored at 4°C in a sterilized bottle for experimental use [10].

Preliminary phytochemical screening

The preliminary phytochemical screening of EEMC was carried out to determine the presence or absence of flavonoids, alkaloids, glycosides saponins, tannins, reducing sugar, steroids as well as anthraquinone as described previously [21, 22].

Insulin-releasing studies *in vitro*

The insulin-releasing effects of EEMC were examined using clonal pancreatic BRIN BD11 β-cells and isolated mouse islets. Clonal pancreatic BRIN BD11 β-cells were incubated with different concentrations (1.6–5000 µg/ml) of EEMC or known insulin secretagogues in the presence of 5.6 mM glucose at 37°C for 20 min [21]. Pancreatic islets were isolated from mice using collagenase P derived from *Clostridium histolyticum* (Sigma-Aldrich, Dorset, UK) and further assayed using insulin radioimmunoassay [21]. Stimulatory concentration of the amino acid alanine (10 mM) and GLP-1 (10^{-6} mM) was used as positive controls [23, 24].

Membrane potential and intracellular calcium ($[Ca^{2+}]_i$)

Using clonal pancreatic BRIN BD11 β-cells, membrane potential (Mp) and intracellular calcium $[Ca^{2+}]_i$ concentrations in the presence of 30 mM KCl, 10 mM alanine, and 200 µg/ml of EEMC were measured (Fig. 2a–d). Ninety-six microplate wells were implemented to grow clonal pancreatic BRIN BD11 β-cells for 18 h at 37°C. Cells were incubated with 100 µl Krebs-Ringer Bicarbonate (KRB) buffer solution at 37°C for 10 min, and further assays were performed by incubating with a FLIPR Membrane Potential and Calcium Assay Kit (Molecular Devices EK0200, Sunnyvale, CA, USA) [21].

Starch digestion *in vitro*

The effects of EEMC on starch digestion were investigated following the previously published method [21]. Starch (Sigma–Aldrich, St. Louis, MO, USA) solution (2 mg/ml, 100 mg in 50 ml) was incubated with or without EEMC (1.6–5000 µg/ml) and acarbose (0.32–1000 µg/ml) in the presence of 0.01% heat stable α-amylase and 0.1% amyloglucosidase (Sigma–Aldrich) at 80°C and at 60°C for 20 and 30 min, respectively. Glucose Oxidase-Peroxidase (GOD-PAP) reagent was used to analyze the glucose liberation using microplate reader [24].

Glucose diffusion *in vitro*

The glucose diffusion *in vitro* model utilized dialysis tubes made of cellulose ester (CE, 20 cm × 7.5 mm, Spectra/Por® CE layer, MWCO: 2000, Spectrum, Amsterdam, the Netherlands). A 2-ml solution of 0.9% NaCl (BDH Chemicals Ltd., Poole, UK) with 220 mM glucose was added to the cellulose ester dialysis tube plus/minus EEMC at a concentration ranging from 200 to 25 000 µg/ml. The tubes were sealed tightly and they were placed in a 50-ml centrifuge tube (Orange Scientific, Orange, CA, USA) filled with 45 ml of 0.9% NaCl. The tubes were shaken on an orbital shaker at 37°C for 24 h, and samples were taken to measure glucose levels [23].

Diphenyl-1-picrylhydrazyl assay *in vitro*

The free radical scavenging activity of EEMC (1.6–5000 µg/ml) was assessed using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method. L-Ascorbic acid (1.6–5000 µg/ml) was used as a standard. A control solution was prepared by adding 2 ml of 0.2 mmol/l DPPH to 1 ml of distilled water. The mixtures were incubated in the dark at room temperature for 30 min. The absorbance was taken using a UV/VIS spectrophotometer (Spectronic 200, Mettler-Toledo, Hamilton, New Zealand) at 517 nm [25].

Animals

This study was conducted on male Sprague–Dawley rats (200–250 g) at the age of 6–8 weeks. The rats were accommodated under optimal conditions at room temperature of 22 ± 5°C, relative humidity of 55%–65%, and a 12-h day/night cycle. A standard rat pellet diet and drinking water were provided ad libitum. To induce diabetes, rats, fasted for 8 h, received an intraperitoneal injection of alloxan monohydrate in 0.9% NaCl solution at a dose of 120 mg/kg body weight. After 1 h of alloxan administration, the animals were fed *ad libitum*. An oral glucose test (OGTT; 2.5 g/kg, b.w) was performed after 72 h of alloxan administration, and rats that manifested blood

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glucose concentrations of 8–12 mmol/l were considered as type 2 diabetes rats. The animal experiments were conducted in accordance with ethical standards. They were approved by the Animal Welfare and Ethical Review Board (AWERB) at Ulster University and carried out under the UK Home Office Animal project/personal license numbers PIL1822 and PPL 2804. The experiments complied with the UK Act 1986 and EU Directive 2010/63EU. All necessary measures were taken to ensure that no animals were harmed during the study.

Feeding test

The food intake was measured in 12 h fasted alloxan-induced diabetic rats at 0, 30, 60, 90, 120, and 180 min after oral gavage of saline (5 ml/kg), EEMC (500 mg/kg), or gliclazide (100 mg/kg), respectively [22].

Acute oral glucose tolerance test

Blood samples were obtained from the tip of the tail of 12 h fasted alloxan-induced diabetic rats at specific time intervals before (0 min) and after (30, 60, 120, and 180 min) oral gavage of glucose (2.5 g/kg) plus/minus EEMC (500 mg/kg) or gliclazide (100 mg/kg), served as the positive control [22].

Gastrointestinal motility test

This study investigated gastrointestinal (GI) motility in alloxan-induced type 2 diabetic rats using barium sulfate ($BaSO_4$) milk as described previously [26]. Rats received EEMC (500 mg/kg), loperamide (5 mg/kg), and sennoside (10 mg/kg) before being fed the $BaSO_4$ milk. The distance traveled by the $BaSO_4$ milk within the small intestine was measured to assess motility [27, 28].

Effects of EEMC on blood glucose, plasma insulin, and lipid profile

The chronic effects of EEMC were assessed by twice daily oral administration of EEMC (500 mg/kg) for 15 days. Control rats received saline water (5 ml/kg) only. Blood samples were collected at an interval of 3 and 6 days from Day 0 to 15 from the tip of the rat tail. Fasting blood glucose levels were measured using a Ascencia Contour glucose meter (Bayer, Newbury, UK), and plasma insulin levels were analyzed using a rat insulin ELISA kit (Crystal Chem, Woburn, MA, USA). The lipid profile such as triglyceride, total cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL), very-low-density lipoprotein (VLDL) and high-density lipoprotein (HDL) were measured using COD-PAP, GPO-PAP (Elabscience Biotechnology Co., Ltd., Houston, TX, USA) and CHOD-PAP (Biolabo SAS, Maizy, France) reagents [10, 22, 29].

Statistical analysis

A statistical analysis software for Windows, Graph Pad Prism 5, was used for all types of analysis of data. All data were analyzed with unpaired Student's *t*-test (parametric, with two-tailed *P*-values), one-way, or two-way ANOVA with Bonferroni post hoc tests (if applicable). Values are expressed as mean ± SEM and the significant limit was determined as $P < 0.05$.

Results

EEMC and insulin secretion

Figure 1a and b illustrates dose-dependent (1.6–5000 µg/ml) insulinotropic effects of EEMC in BRIN-BD11 cells. At 5.6 mM glucose, basal insulin secretion from BRIN BD11 cells was 0.65 ± 0.14 ng/10^6 cells/20 min, which increased to 4.33 ± 0.28 ng/10^6 cells/20 min with insulin secretagogues,10 mM alanine ($P < 0.001$; Fig. 1a). EEMC (40–5000 µg/ml) showed concentration-dependent increase in insulin release from 1.64 ± 0.27 to 3.71 ± 0.41 ng/10^6 cells/20 min ($P < 0.01$–0.001; Fig. 1a) with 5.6 mM glucose. In the presence of 16.7 mM glucose, basal insulin release from BRIN-BD11 cells was found to be 1.02 ± 0.12 ng/10^6 cells/20 min, whereas in the presence of positive control, KCl (30 mM), it reached to 7.43 ± 0.80 ng/10^6 cells/20 min ($P < 0.001$; Fig. 1b). Similarly, the dose-dependent (40–5000 µg/ml) insulin-releasing effects of EEMC from BRIN-BD11 were observed with significantly increased insulin release from 2.17 ± 0.20 to 6.42 ± 0.35 ng/10^6 cells/20 min ($P < 0.05$–0.001; Fig. 1b) in the presence of 16.7 mM glucose.

The dose-dependent (25–200 µg/ml) insulin-releasing effects of EEMC from isolated mouse islets are depicted in Fig. 1c. At 16.7 mM glucose, the basal rate of insulin secretion from isolated mouse islets was found to be 3.82 ± 0.55 ng/10^6 cells/20 min which was significantly stimulated by positive controls, alanine (10 mM) and GLP-1 (10^{-6}), to 16.73 ± 2.27 and 23.14 ± 1.78 ng/10^6 cells/20 min, respectively ($P < 0.001$; Fig. 1c). Similarly, EEMC exhibited significant dose-dependent (50–200 µg/ml) insulin releasing effects on isolated mouse islets from 8.18 ± 0.77 to 14.03 ± 1.22 ng/10^6 cells/20 min ($P < 0.05$–0.001; Fig. 1c) in the presence of 16.7 mM glucose.

The insulin secretory actions of EEMC (200 µg/ml) were further assessed using insulin modulators (Fig. 1d). Modulators such as 16.7 mM glucose, IBMX, and tolbutamide enhanced insulin release from BRIN BD11 cells ($P < 0.05$–0.001, Fig. 1d). With 200 µM IBMX, 200 µM tolbutamide, and a depolarizing concentration of 30 mM KCl, EEMC ameliorated insulin secretion by 1.4, 1.7, and 1.1 folds ($P < .001$; Fig. 1d), respectively. However, the insulin-releasing rate was reduced by 22%–25% in the presence of the K^+ channel activator, 300 µM diazoxide, and the L-type voltage-dependent Ca^{2+} channel blocker, 50 µM verapamil ($P < 0.05$; Fig. 1d).

EEMC and Mp, and $[Ca^{2+}]_i$ in clonal pancreatic BRIN-BD11 β-cells

Membrane depolarization and intracellular calcium ($[Ca^{2+}]_i$) concentration were measured in clonal pancreatic BRIN-BD11 β-cells (Fig. 2a–d). Incubation of BRIN-BD11 cells with a depolarizing concentration of 30 mM KCl and insulin secretagogues, 10 mM Alanine resulted in a substantial increase in Mp ($P < 0.001$; Fig. 2a) and an induce in $[Ca^{2+}]_i$ concentrations ($P < 0.001$; Fig. 2c). Area under the curve showed that 30 mM KCl depolarized membrane by 96% (Fig. 2b) whereas 10 mM alanine increased $[Ca^{2+}]_i$ concentration by 93% (Fig. 2d) at 5.6 mM glucose. EEMC, at a dose of 200 µg/ml, significantly ($P < 0.01$–0.001) produced Mp and improved $[Ca^{2+}]_i$ concentration (Fig. 2a–d) at 5.6 mM glucose and area under the curve depicted a 71% increase in Mp (Fig. 2b) and 57% rise in $[Ca^{2+}]_i$ concentration (Fig. 2d).

EEMC and starch digestion *in vitro*

Figure 2e and f demonstrates the effects of acarbose and EEMC on starch digestion. The positive control, acarbose (1.6–1000 µg/ml), showed 16%–78% ($P < 0.01$–0.001; Fig. 2e) dose-dependent inhibitory effect on glucose liberation

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from starch whereas EEMC at a dose of 40–5000 µg/ml exhibited 8%–20% reduction in enzymatic glucose liberation from starch ($P < 0.05$–0.001; Fig. 2f).

EEMC and glucose diffusion *in vitro*

The effects of EEMC on glucose diffusion *in vitro* were observed at the interval of 0, 3, 6, 12, and 24 h incubation, respectively. EEMC significantly decreased glucose diffusion/absorption in a time and dose-dependent (200–25 000 µg/ml) manner. At 0 h, no significant changes were noticed (Fig. 3a). At 3, 6, and 12 h, EEMC significantly inhibited glucose absorption with inhibition rate ranges from 9.62% to 18.38%, 9.88% to 27.83%, and 13.55% to 29.33% ($P < 0.05$–0.001; Fig. 3b–d), respectively, in the concentration (1000–25 000 µg/ml) dependent manner. At 24 h, the concentrations (200–25 000 µg/ml) of EEMC were found to be more effective in inhibiting glucose absorption from 13.37% to 31.08% ($P < 0.05$–0.001; Fig. 3e).

EEMC and DPPH assay *in vitro*

Table 1 demonstrates the concentration-dependent DPPH scavenging activity of positive control L-ascorbic acid (1.6–5000 µg/ml), which showed 14.17%–97.08% ($P < 0.01$–0.001; Table 1) DPPH inhibition and EEMC (8–5000 µg/ml), exhibited 8.71–77.10% ($P < 0.05$–0.001; Table 1) DPPH inhibition.

EEMC and feeding test

Figure 4a represents the food intake measured during the acute feeding test in alloxan-induced type 2 diabetic rats. EEMC, orally administered at a dose of 500 mg/kg, significantly reduced food intake at 30 and 60 min ($P < 0.05$, $P < 0.001$); Fig. 4a). Similarly, the positive control, sulfonylurea drug, gliclazide (GCZ) at a dose of 100 mg/kg, showed a consistent time-dependent decrease in food intake ($P < 0.05$–0.001; Fig. 4a) when compared with alloxan-induced type 2 diabetic control rats (DC).

EEMC and acute oral glucose tolerance test

The oral administration of glucose (18 mmol/kg, b.w) with EEMC (500 mg/ kg) exhibited a significant improvement in oral glucose tolerance at 30 and 60 min in alloxan-induced type 2 diabetic rats ($P < 0.05$, $P < 0.001$; Fig. 4b). The positive control, gliclazide (100 mg/kg), a sulfonylurea drug, ameliorated glucose tolerance ($P < 0.01$–0.001; Fig. 3b) in a time-dependent manner.

EEMC and GI motility

Figure 3c illustrates that the oral gavage of EEMC at a dose of 500 mg/kg resulted in a significant improvement in GI motility ($P < 0.05$; Fig. 4c). In contrast, the antidiarrheal drug, loperamide (5 mg/kg), decreased gut motility ($P < 0.05$; Fig. 4c), while the stimulant laxative bisacodyl (5 mg/kg) promoted gut motility ($P < 0.01$; Fig. 4c) compared with alloxan-induced DC.

EEMC and blood glucose, plasma insulin, and lipid profile

The twice daily oral gavage of EEMC (500 mg/kg) in alloxan-induced type 2 diabetic rats resulted in a consistent reduction of non-fasting blood glucose levels, 15.20 vs 12.69 ($P < 0.01$, DC vs DC + EEMC, Day 3), 15.41 vs 10.49 ($P < 0.001$, DC vs DC + EEMC, Day 9), and 15.70 vs 9.49 ($P < 0.001$, DC vs DC + EEMC, Day 15) mmol/l (Fig. 4d), respectively. The positive control, gliclazide (100 mg/kg), also attenuated non-fasting blood glucose level in a time-dependent manner, 15.20 vs 10.66 ($P < 0.001$, DC vs DC + GCZ, Day 3), 15.41 vs 9.52 ($P < 0.001$, DC vs DC + GCZ, Day 9), 15.70 vs 6.58 ($P < 0.001$, DC vs DC + GCZ, Day 15) mmol/l (Fig. 4d), respectively. EEMC at a dose of 500 mg/kg also improved plasma insulin levels in alloxan-induced type 2 diabetic rats ($P < 0.01$; Fig. 4e) which was comparable with the positive control gliclazide, a sulfonylurea drug, that possessed a significant improvement in plasma insulin level ($P < 0.001$; Fig. 4e).

The impact of EEMC on lipids is presented in Fig. 5, revealing a significant amelioration in blood lipid profile with a decrease in circulating triglyceride ($P < 0.05$; Fig. 5a), total cholesterol ($P < 0.01$; Fig. 5b), LDL ($P < 0.01$; Fig. 5c), and VLDL cholesterol levels ($P < 0.001$; Fig. 5d) as well as a marked increase in HDL cholesterol level ($P < 0.001$; Fig. 5e) compared with diabetic control (DC). Similarly, the positive control gliclazide improved circulating triglycerides, total cholesterol, HDL, LDL, and VLDL levels ($P < 0.01$–0.001; Fig. 5a–e), respectively.

Preliminary phytochemical screening of EEMC

The preliminary phytochemical screening of EEMC identified the presence of alkaloids, tannins, saponins, steroids, glycosides, and flavonoids (Table 2) through which it may possess its antihyperglycemic as well as antihyperlipidemic effects.

Discussion

The search for safer and more economical oral antihyperglycemic drugs has led to the exploration of medicinal plants and their phytoconstituents for the management of T2DM. The consumption of *M. charantia*, also known as bitter gourd, is a promising therapy for lowering blood glucose levels, increasing insulin secretion, and reducing insulin resistance [14, 16–19]. This study aims to investigate the mechanism of action of EEMC by exploring its insulin-releasing and glucose-lowering actions through *in vitro* and *in vivo* studies.

In this study, the investigation of the insulinotropic effects of EEMC *in vitro* was conducted using clonal pancreatic BRIN-BD11 β-cells and isolated mouse islets. EEMC showed a dose-dependent increase in insulin release with 5.6 and 16.7 mM glucose, respectively. Alanine and GLP-1 were used as positive controls to validate the insulinotropic effects of EEMC in both BRIN-BD11 cells and isolated mouse islets as both are strong insulin modulators that show insulin secretory action in response to glucose via inactivation of ATP-sensitive K^+ channel and subsequent depolarization of the plasma membrane and increase in intracellular Ca^{2+} [21].

The impact of a nontoxic dose of EEMC was assessed using insulin-releasing or inhibiting modulators to better comprehend the mode of action of insulin secretion. EEMC increased insulin release from clonal pancreatic BRIN-BD11 β-cells with ATP-sensitive K+ channel (K_{ATP}) channel blocker, tolbutamide, and a depolarizing concentration of 30 mM KCl. Consequently, this shows that the EEMC may stimulate insulin secretion via a variety of mechanisms, including direct effects on exocytosis, the PI3 (phosphatidylinositol pathway), or the adenylate cyclase or cyclic adenosine monophosphate

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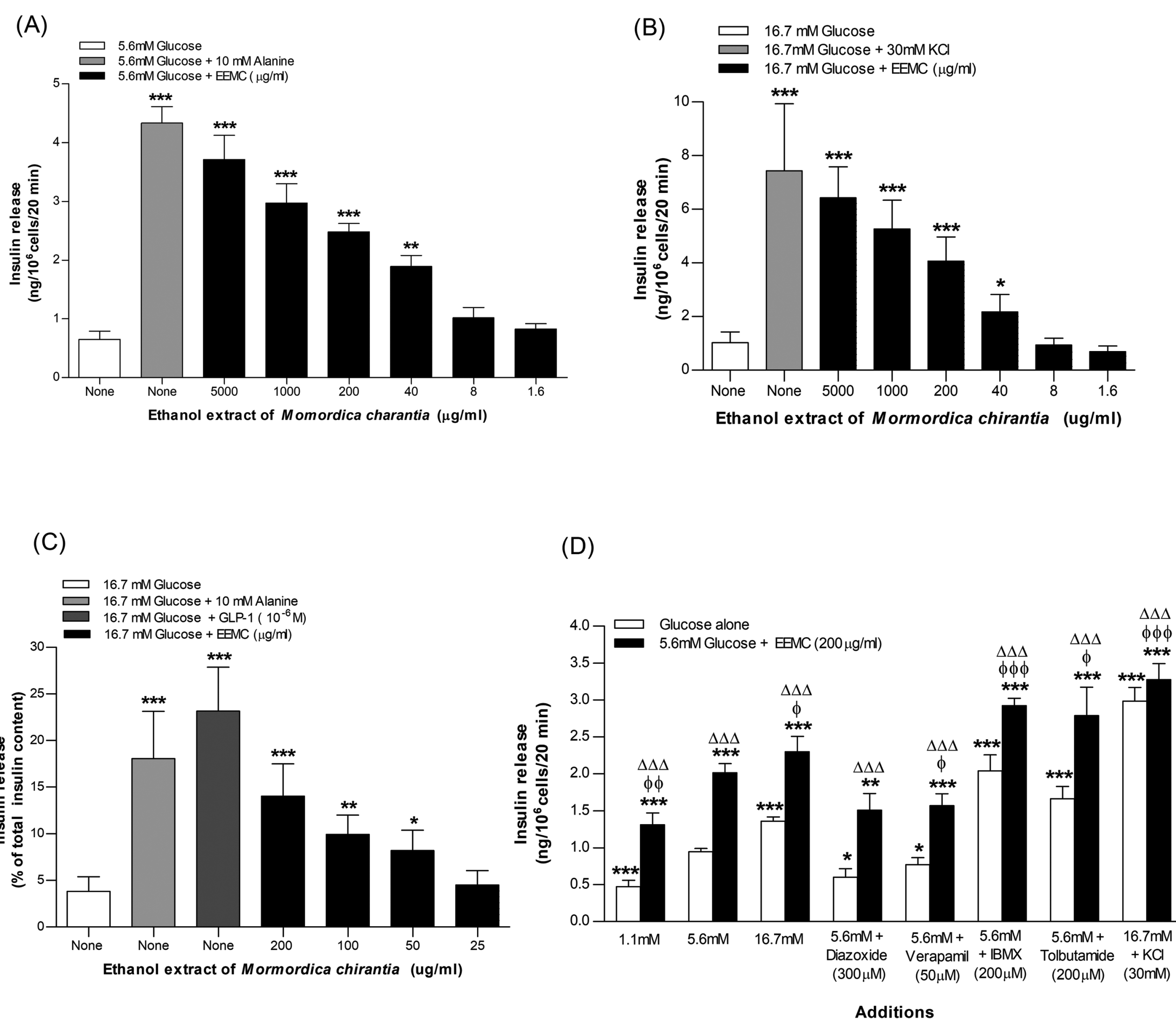

Figure 1. Dose-dependent effects of EEMC on insulin release from clonal pancreatic BRIN BD11 β-cells at (a) 5.6 mM glucose, (b) 16.7 mM glucose, (c) isolated mouse islets, and (d) insulin secretion with known stimulators or inhibitors. The BRIN-BD11 cells and isolated mouse islets were incubated with or without insulin secretagogues and EEMC plus or minus glucose (5.6 and 16.7 mM). Values, *n* = 4–8, for insulin release are expressed as mean ± SEM. $^{*,**,***}P < 0.05$–0.001 compared with control (5.6/16.7 mM glucose). $^{\phi,\phi\phi,\phi\phi\phi}P < 0.05$–0.001 compared with 5.6 mM glucose with EEMC for (d). $^{\Delta\Delta\Delta}P < 0.001$ compared with respective incubation without EEMC for (d). EEMC, ethanolic extract of *M. charantia*.

(cAMP) pathway [30]. Verapamil, a voltage-dependent Ca^{2+} channel blocker, partly reduced EEMC's ability to secrete insulin, further supporting the hypothesis that K_{ATP} channel opener is also involved in the partial reduction of EEMC's ability to release insulin in response to diazoxide [23]. In clonal pancreatic BRIN-BD11 β-cells, EEMC produced membrane depolarization and elevated intracellular calcium levels, suggesting that EEMC may potentiate its insulin stimulatory effects via K_{ATP} and Ca^{2+} channel-dependent pathways [24, 30]. Insulin secretion triggered significantly when IBMX, a cAMP phosphodiesterase inhibitor, was incubated with EEMC, demonstrating the cAMP pathway's involvement [30]. In lung tissues, *M. charantia* can lead to a rise in cAMP, which attenuates the growth of bronchial smooth muscle cells and improves airway relaxation [31].

Postprandial hyperglycemia, high blood sugar after meals, is known as a potential risk factor for cardiovascular disease&& (CVD). Therefore, treating postprandial hyperglycemia could help prevent secondary cardiovascular complications in people with type 2 diabetes [32]. One way to manage postprandial hyperglycemia is to inhibit the activity of α-amylase and α-glucosidase. These enzymes break down carbohydrates into glucose, which can contribute to high blood sugar levels after eating [33]. The present studies showed that EEMC effectively inhibits the breakdown of starch in a concentration-dependent manner. Additionally, previous studies stated that the flavonoid contents of *M. charantia* such as rutin, epicatechin, naringin, genistein, and naringenin are effective against α-amylase and delay starch digestion [34]. Moreover, consumption of dietary fiber can restrain hunger as it obstructs stomach evacuation and postpones the assimilation of energy and nutrients. Thus, the high fiber content of *M. charantia* may also contribute to delaying digestion and prolonging nutrient absorption, potentially leading to postprandial glucose reduction [35–37].

Soluble dietary fibers are known to have an impact on blood glucose levels in the body. Both the molecular mass and concentration of these fibers play a crucial role in

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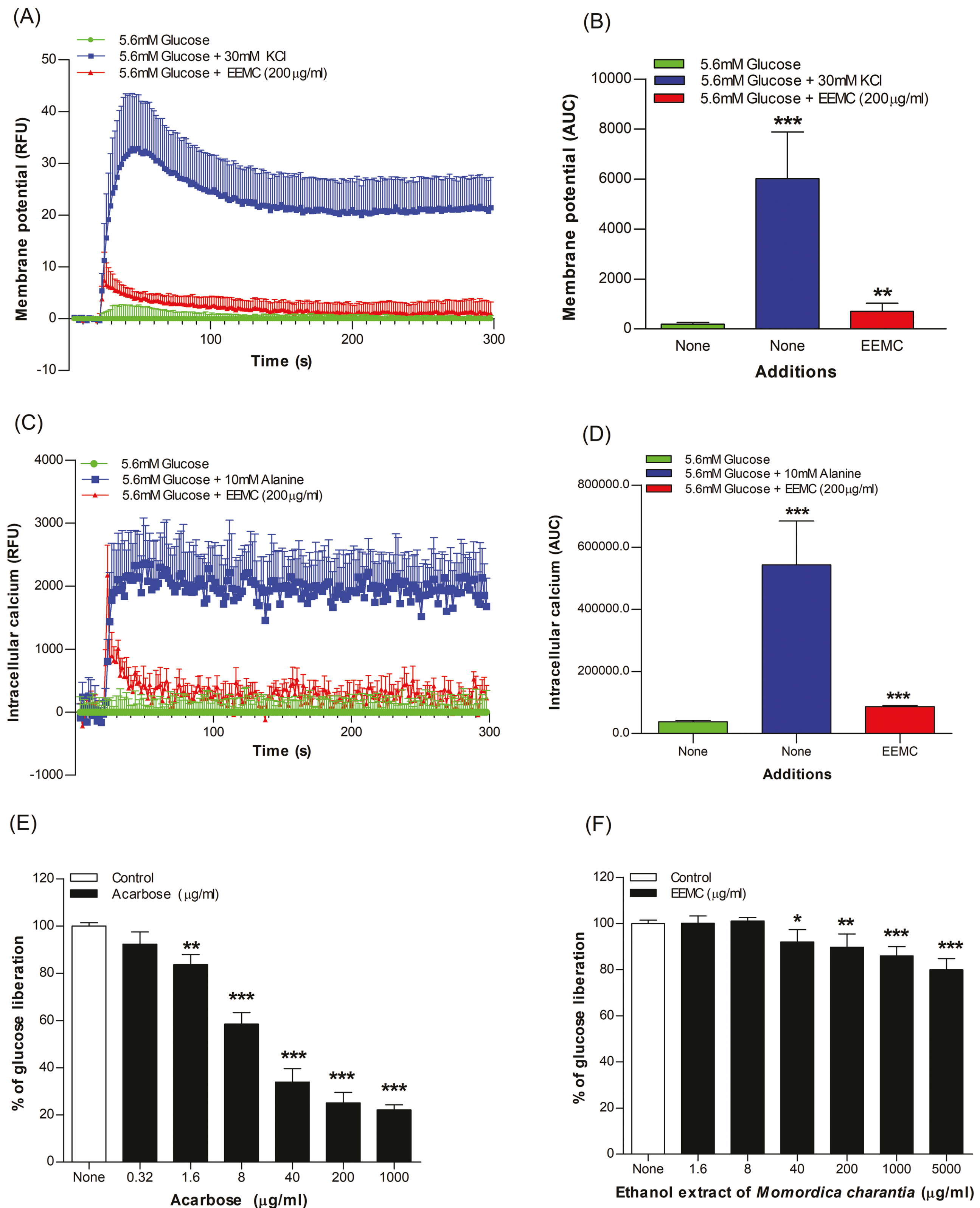

Figure 2. Effects of EEMC on (a, b) Mp and (c, d) intracellular calcium in clonal pancreatic BRIN BD11 β-cells, and starch digestion *in vitro* using (e) acarbose and (f) EEMC. The starch digestion was performed with or without acarbose (0.32–1000 µg/ml) and EEMC (1.6–5000 µg/ml) followed by incubation with 0.01% α-amylase and 0.1% amyloglucosidase. Values, $n = 6$, for Mp and intracellular calcium, $n = 4$, for starch digestion are expressed as mean ± SEM. *,**,***$P < 0.05$–0.001 compared with control.

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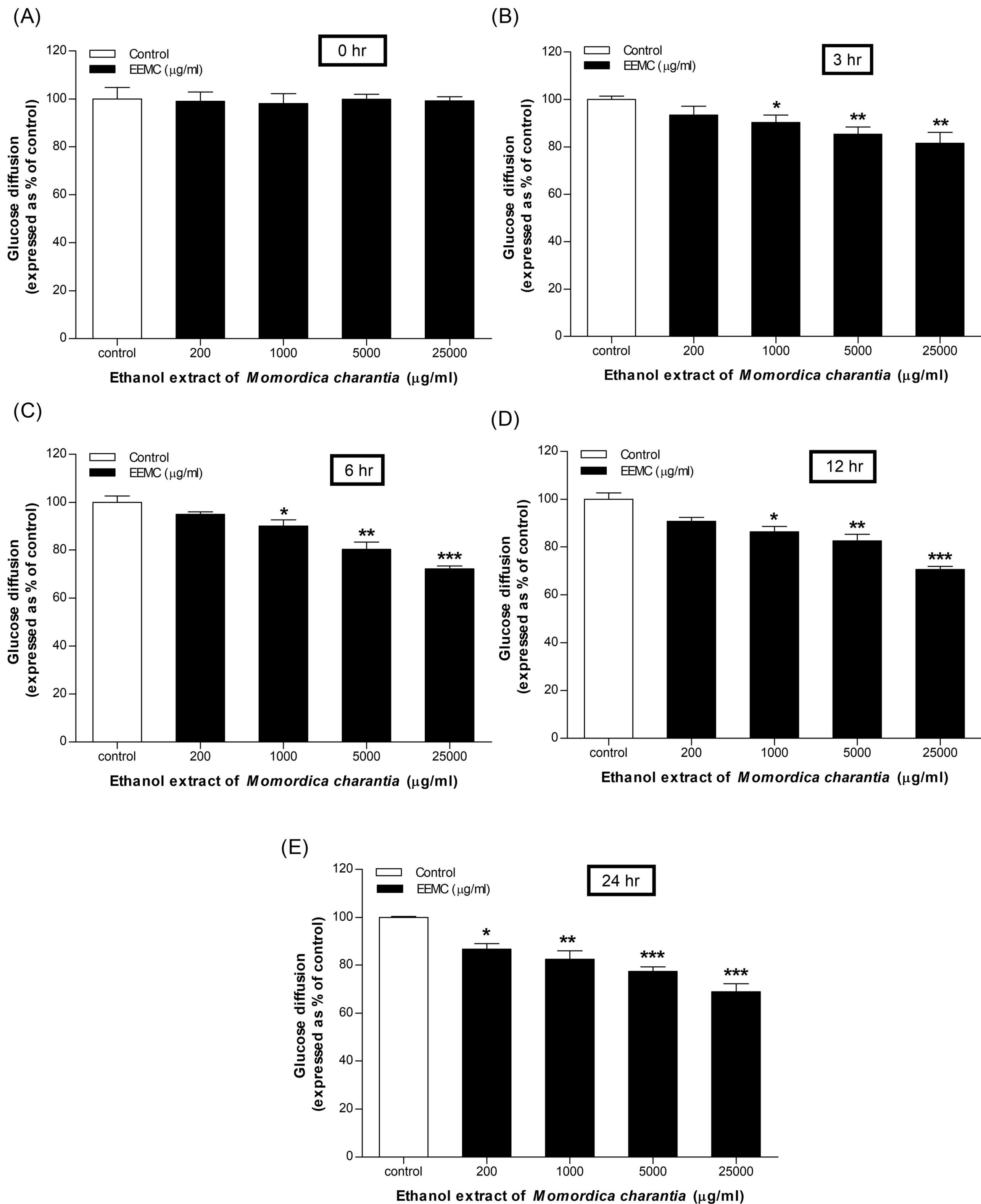

Figure 3. Dose-dependent effects of EEMC on (a–d) glucose diffusion *in vitro*. The experiment was conducted with or without EEMC (200–25 000 μg/ml) using dialysis tubing cellulose membrane and the diffusion of glucose were measured at 0, 3, 6, 12, and 24 h, respectively, followed by the GOD-PAP method. Values, $n = 4$, for glucose diffusion are expressed as mean ± SEM. *,**,***$P < 0.05$–0.001 compared with control.

their glucose-lowering actions [2]. In this study, we employed an *in vitro* dialysis-based model to examine the effects of EEMC on glucose diffusion. The dialysis model we employed involved continuous agitation to mimic the movement observed in the GI tract. However, it is important to note that this model has certain limitations. It does not directly correlate the temporal dynamics of cellular mechanisms responsible for glucose absorption in the gut with

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the time required for glucose to fully diffuse across the dialysis membrane (22–26 h) [36, 38]. We found that EEMC exhibited a dose-dependent inhibition of glucose absorption. These findings are consistent with previous studies that have suggested that *M. charantia* may inhibit glucose absorption by suppressing the activity of intestinal maltase, pancreatic lipase, and sucrase.

Table 1. Antioxidant activity of L-ascorbic acid and EEMC using DPPH scavenging assay techniques.

Concentration (µg/ml)	Ascorbic acid (% inhibition)	EEMC (% inhibition)
1.6	14.17 ± 2.04**	4.492 ± 2.01
8	34.71 ± 1.94***	8.709 ± 1.94*
40	65.29 ± 2.14***	13.90 ± 2.33**
200	87.05 ± 1.61***	25.79 ± 2.08**
1000	95.19 ± 1.11***	55.00 ± 2.01***
5000	97.08 ± 2.09***	77.10 ± 2.01***

Dose-dependent effects of DPPH scavenging activity of L-ascorbic acid and EEMC. Test was carried out with (treatment) or without (control) L-ascorbic acid and EEMC at different concentrations ranging from 1.6 to 5000 µg/ml followed by 30 min incubation under a dark room condition. DPPH inhibition were expressed as % of control. Values, n = 3, are expressed as mean ± SEM. *, **, ***P < 0.05–0.001 compared with control.

Recent evidence suggest that oxidative stress plays a crucial role in the pathogenesis of diabetes, whereby non-enzymatic protein glycation, increased lipid peroxidation, and glucose oxidation trigger free radical formation, leading to damage of cellular machinery, enzymes, and insulin resistance [39]. EEMC exhibited a significant dose-dependent inhibition of DPPH. Recent studies reported that antioxidant content of natural resources such as catechin, isoquercitrin, quercetin, and kaempferol can scavenge the free radicals and reduce oxidative stress [6], indicating that EEMC may reduce the oxidative stress due to the presence of following flavonoids [39, 40].

Over consumption of foods also elevates the risk of obesity, insulin resistance, and T2DM [41]. The feeding test was conducted to assess the effects of EEMC on food intake in alloxan-induced type 2 diabetic rats. Both EEMC and positive control gliclazide treatments displayed a substantial decrease in food intake. Previous studies stated that due to the presence of lectin in *M. charantia* may exhibit the appetite-suppressing effect followed by reduced food intake [42].

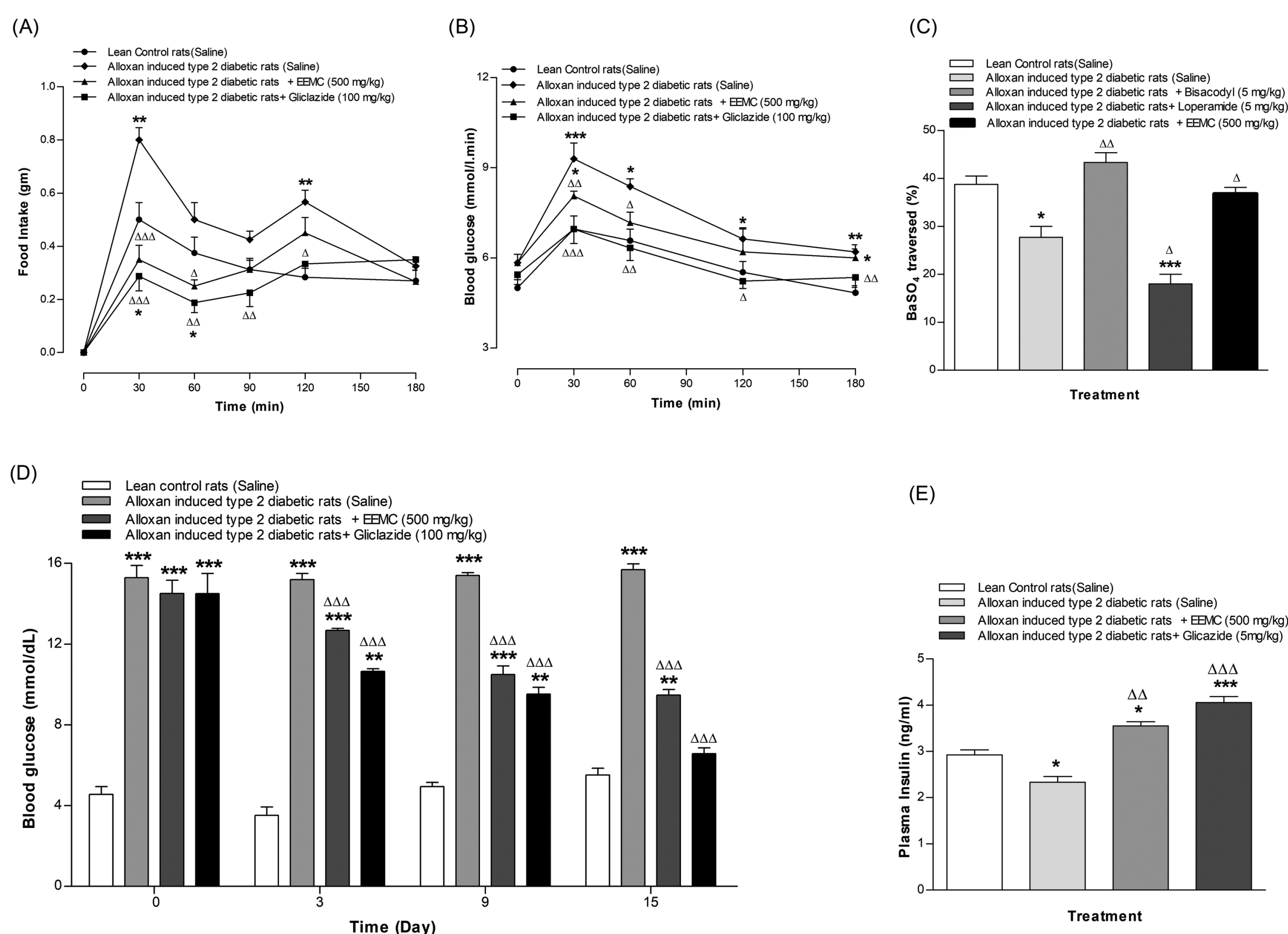

Figure 4. Effects of EEMC on (a) food intake during acute feeding test, (b) glucose tolerance, (c) GI motility (by $BaSO_4$ traversed), (d) non-fasting blood glucose, and (e) plasma insulin in alloxan-induced type 2 diabetic rats. Tests were performed following 15 days twice-daily administration of EEMC (500 mg/kg, b.w.) and gliclazide (100 mg/kg, b.w.) in alloxan-induced type 2 diabetic rats. The GI motility was measured by calculating the percentage of distance traveled by $BaSO_4$ milk via intestine. Blood glucose and plasma insulin levels were measured using a Ascencia Contour glucose meter and insulin ELISA kit, respectively. Values, n = 6, for feeding test, glucose tolerance, GI motility, blood glucose, and plasma insulin are expressed as mean ± SEM. *,**,***P < 0.05–0.001 compared with lean control and Δ,ΔΔ,ΔΔΔP < 0.05–0.001 compared with alloxan-induced type 2 diabetic rats.

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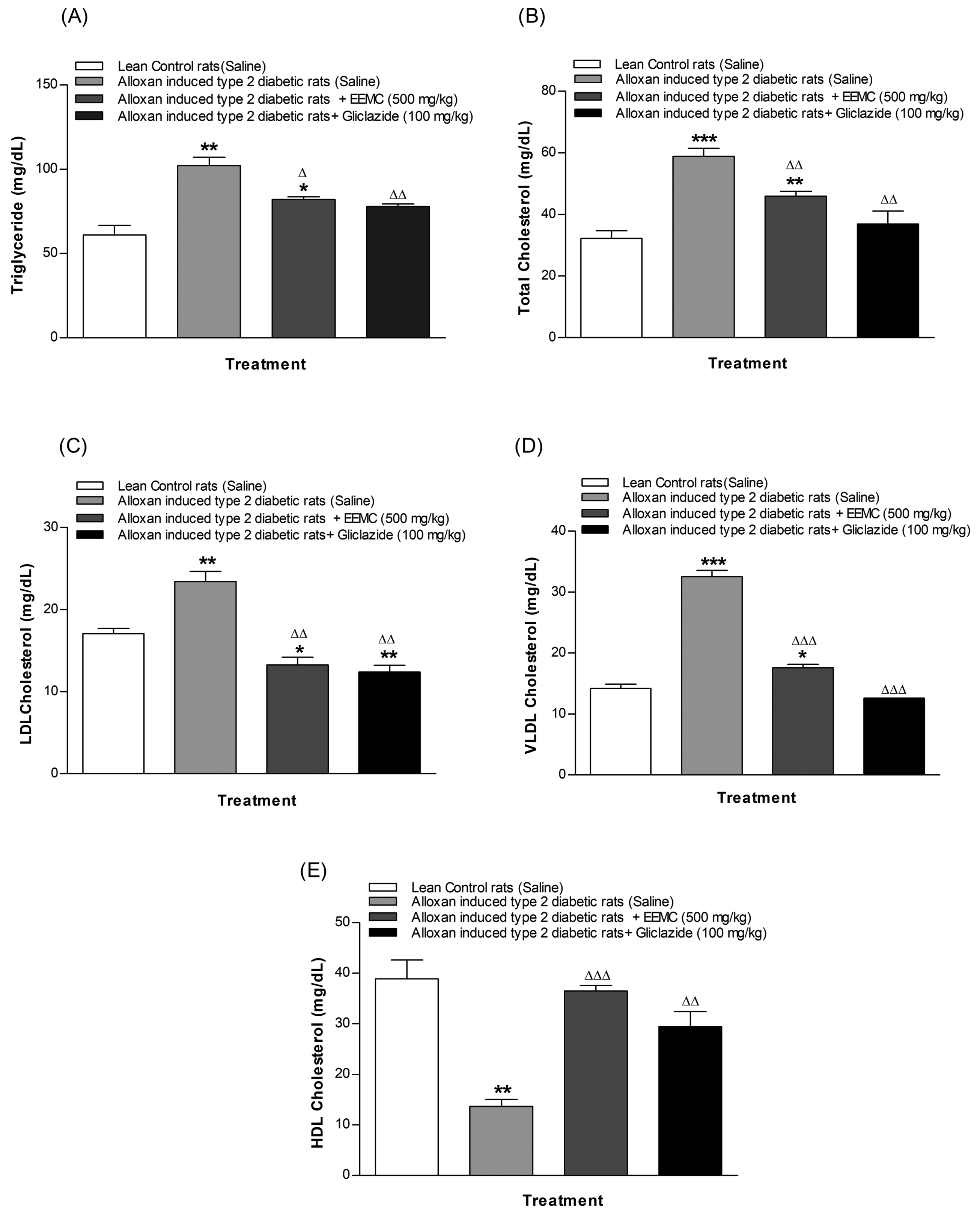

Figure 5. Effects of EEMC on circulating (a) triglyceride, (b) total cholesterol, (c) LDL, (d) VLDL, and (e) HDL cholesterol in alloxan-induced type 2 diabetes rats. At the end of studies, the blood samples were collected and centrifuged to separate the plasma serum, and lipid profiles such as triglyceride, total cholesterol, LDL, VLDL, and HDL cholesterol, were measured using COD-PAP, GPO-PAP, and CHOD-PAP reagents, respectively. Values, $n = 6$, are expressed as mean ± SEM. $^{*,**,***}P < 0.05$–0.001 compared with control and $^{\Delta,\Delta\Delta,\Delta\Delta\Delta}$ $P < 0.05$–0.001 compared alloxan-induced type 2 diabetic rats.

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Table 2. Preliminary phytochemical screening of EEMC.

Phytochemical group test	Observation
Alkaloid	+
Tannin	+
Saponin	+
Steroid	+
Glycoside	+
Flavonoid	+
Reducing sugar	–
Anthraquinone	–

The '+' sign indicating the presence of phytoconstituents while '–' sign indicating the absence of phytoconstituents in ethanolic extract of *Momordica charantia*. The test was replicated three times with $n = 3$.

There are several strategies that can be implemented to prevent or delay the onset of diabetes in individuals with impaired glucose tolerance or fasting blood glucose [43]. An acute oral glucose tolerance test was performed in alloxan-induced type 2 diabetic rats where both EEMC and standard drug gliclazide demonstrated a significant amelioration in glucose tolerance. These results are consistent with previous studies, suggesting that *M. charantia* may have a direct effect on the disposal of glucose in the liver or the periphery, thereby lowering blood sugar levels [44].

Hyperglycemia, or high blood sugar levels, can have negative effects on gastric motility or the movement of food from the stomach into the small intestine in individuals with diabetes. This is due to the development of irreversible autonomic neuropathy, which affects the nerves that control the function of the stomach [45]. EEMC exhibited a significant increase in intestinal motility. Dietary fibers play a key role in mediating these actions. They can affect the viscosity and movement of food in the GI tract [2]. As a result, *M. charantia*, with its high dietary fiber content, may reduce glucose absorption and increase gut motility. This is believed to be one of the reasons for its ability to exhibit these effects [2, 3].

In our study, we found that 15 days of twice-daily oral administration of EEMC significantly reduced non-fasting blood glucose levels in a time-dependent manner and improved plasma insulin levels in alloxan-induced type 2 diabetic rats. Previous studies reported that *M. charantia* can lower blood glucose levels and improve plasma insulin levels in streptozotocin-induced diabetic rats [46, 47]. This effect of *M. charantia* may be due to the restoration and proliferation of insulin-producing cells, as well as enhanced insulin secretion [35]. The mechanisms that may underlie the hypoglycemic effects of EEMC include increased phosphorylation of the insulin receptor substrate and activation of AMPK activity [47, 48].

The lipid profile studies of the EEMC showed a significant reduction in plasma triglyceride, total cholesterol, LDL, and VLDL levels, along with a notable increase in HDL levels. These findings are consistent with a previous 30-day study of methanol extract of *M. charantia*, which showed that the improvement in lipid profile could be attributed to the potentiating effects on lipoprotein catabolism [49]. Additionally, the increase in HDL levels may be attributed to the high thyroxine level, which is known to facilitate the elimination of LDL from circulation by transporting it to the liver [49].

The preliminary phytochemical screening of EEMC revealed the presence of alkaloids, tannins, saponins, steroids, glycosides, and flavonoids which may possess antidiabetic properties. Vicine, a glycol alkaloid in *M. charantia*, lowers blood glucose in rats [20]. Tannins aid in glucose uptake and suppress adipogenesis [50]. Steroidal saponin charantin exhibits insulin-like properties [51], cucurbitacin B lowers blood glucose via the AMPK pathway [52], while flavonoids like catechin Epicatechin, *p*-coumaric acid, and cinnamic acid improve various diabetic parameters including hyperglycemia and insulin resistance [53, 54].

Conclusion

This study provides strong evidence that *M. charantia* could be a promising alternative treatment for hyperglycemia. The study found that EEMC increased insulin secretion, offered antioxidant benefits, and improved blood glucose, plasma insulin, and lipid levels. Our studies emphasize that insulin-releasing properties of EEMC may be mediated by closing the K_{ATP} channel and opening the Ca^{2+} channel or through a secondary messenger pathway. These findings suggest that EEMC could be a beneficial nutritional supplement for managing type 2 diabetes mellitus and propose further studies to confirm its safety and efficacy in humans.

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Author contributions

P.A., P.R.F., and Y.H.A.A.-W. conceived and designed the research study, and equally supervised it. P.A., J.T.K., M.S., L.H., S.C., S.K.P., and S.R.P. conducted the experiments and analyzed the data. P.A. and J.T.K. interpreted the results, prepared the figures, and drafted the manuscript with Y.H.A.A.-W, P.A. and Y.H.A.A.-W edited the revised manuscript. All authors read and approved the final manuscript.

Conflict of interest statement

The authors declare no conflict of interest.

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This study was conducted without any external funding.

Data availability

The data reported in this study are not publicly available due to some restrictions. However, the corresponding author can provide the data upon reasonable request.

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