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Original Research

Acute Anti-Hyperglycaemic Activity of Five Traditional Medicinal Plants in High Fat Diet Induced Obese Rats

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Abstract

Background: Diabetes mellitus (DM), a prevalent non-communicable disease, is a metabolic condition involving defective pancreatic β-cells and/or insulin resistance. Researchers are presently exploring traditional medicinal plants to identify alternatives for treating diabetes due to the various disadvantage of current anti-diabetic medicines. **Objective**: The present study evaluated the anti-hyperglycaemic effects of ethanol extracts of five medicinal plants (EEMPs) (*Gynura nepalensis*, *Glochidion thomsonii*, *Clerodendrum splendens*, *Clerodendrum infortunatum* and *Xanthium strumarium*) which are traditionally used as an ethnomedicine to treat diabetes and numerous other health problems. **Methods**: High-fat fed (HFF) obese rats were used to perform acute *in vivo* tests, including oral glucose tolerance, feeding test, metabolic studies, and gastrointestinal motility using BaSO4 milk solution. Priliminary phytochemical screening were performed to discover the presence or absence of alkaloids, tannins, saponins, steroids, glycosides, flavonoids, and reducing sugars in extracts. **Results**: Oral administration of ethanol extracts (250 mg/kg, body weight), along with glucose (18 mmoL/kg body weight), ameliorated glucose tolerance ($p < 0.05$–0.01). In addition, the extracts improved gut motility (250 mg/kg; $p < 0.05$–0.001), as well as reduced food intake during the feeding test (250 mg/kg; $p < 0.05$–0.001). Phytochemical screening of these medicinal plants depicted the presence of flavonoids, alkaloids, tannins, saponins, steroids and reducing sugars. **Conclusions**: Phytochemicals such as flavonoids, tannins and saponins may be responsible for the glucose-lowering properties for these plants. Additional research is warranted to fully identify the bioactive phytomolecules and mechanistic pathways that might lead to the development of a viable, cost-effective type 2 diabetes therapy.

Keywords: traditional medicine; phytoconstituents; diabetes; glucose; gut motility

1. Introduction

Mortality rates in diabetes mellitus (DM) are consistently rising across the globe, affected approximately 10% of the adult population worldwide [1]. DM is characterized as a chronic disease resulting from insulin resistance, inadequate insulin production or both. The three main categories of diabetes are: type 1, type 2, and gestational diabetes [2]. Type 1 diabetes mellitus (T1DM) also known as insulin-dependent diabetes, accounts for 10% of all diabetic patients and is distinguished by the almost total loss of pancreatic β-cells [3]. Type 2 diabetes mellitus (T2DM) or non-insulin-dependent diabetes mellitus, accounts for 90% of all diabetic patients and is classified as defective insulin signalling and/or insufficient production of insulin. Obesity, physical inactivity, genetic factors, and chronic hyperglycaemia are the main contributors of T2DM [4]. Chronic hyperglycaemia associated with T2DM results in a number of secondary conditions including both macro and microvascular complications, and without proper treatment these may result in cardiac arrest, stroke, blindness, and renal failure [5,6].

The initial steps in managing T2DM are a proper diet, weight management, as well as regular physical activity [7]. In addition to these, various medication categories, including metformin, sulphonylureas, thiazolidinediones, Glucagon-like peptide 1 (GLP-1) analogues, Glucose-dependent Insulinotropic Peptide (GIP) and GLP-1R co-agonists, dipeptidyl peptidase-IV (DPP-IV) inhibitors, sodium-glucose co-transporter 2 (SGLT2) inhibitors and synthetic insulin, are now used as treatments for T2DM [8]. However, these synthetic medications come with a host of negative side effects, such as gastrointestinal abnormalities, obesity, hepatic and renal disorders, low blood sugar level, and are often expensive and unavailable to rural and economically deprived areas [4,9,10]. As a result, researchers are devoting a great deal of time and energy into studying plants and other natural sources in an effort to discover new treatments to combat diabetes mellitus [11]. Since ancient times, medicinal plants, such as *Gynura nepalensis* DC., *Glochidion thomsonii* (Müll.Arg.) Hook.f., *Clerodendrum splendens* G.Don, *Clerodendrum infortunatum* L. and *Xanthium strumarium* L. have been employed in local communities to treat illnesses, such as diabetes, hy-

pertension, liver disease, lung problems, microbial infections, cancer, inflammation, and gastrointestinal problems, due to their numerous health benefits [12–16]. These plants contain a variety of potent bioactive phytoconstituents, including rutin, quercetin, linolenic acid, epicatechin, gymnemic acid, kaempferol, berberine, gallic acid and iminosugars which is particularly present in *Casuarina equisetifolia* L., are capable of reducing high blood sugar levels by increasing insulin secretion, inhibiting glycosidase and glycosyl transferase activity as well as protecting pancreatic β-cells from damage, inhibiting DPP-IV enzyme activity and thus, these compounds may aid researchers to discover and develop alternative therapies to treat T2DM [17–22].

The aim of the current study was to evaluate glucose lowering properties of five traditional medicinal plants (*X. strumarium*, *C. infortunatum*, *C. splendens*, *G. thomsonii* and *G. nepalensis*) including the effects on food intake and gut motility *in vivo*.

2. Material and Methods

2.1 Collection and Preparation of Plant Extracts

The five plants, *X. strumarium* (leaves), *C. infortunatum* (roots), *C. splendens* (leaves), *G. thomsonii* (bark) and *G. nepalensis* (leaves) were obtained from Jahangirnagar University, Dhaka, Bangladesh, and a plant taxonomist from Bangladesh National Herbarium identified and allocated their individual accession numbers DACB87271, DACB87272, DACB87273, DACB87274 and DACB87275 respectively. Following rinsing and air drying of the plant parts, 200 g of the dried plant powder was added to 1 liter of 80 % (v/v) ethanol and shaken at a speed of 900 g for 48 to 72 h at room temperature. The mixture was separated with filter paper (Whatman no. 1), and a rotary evaporator (BibbyRE-200, Sterilin Ltd., Newport, UK) was then used to dehydrate the filtered extract. The final product was lyophilized in a freeze dryer vacuum (Savant Speed vac, New York, NY, USA), and then preserved at 4 °C for further experiments [23].

2.2 Animals

Six to eight weeks old Long Evan male rats (200–250 g) were fed a high-fat diet (20% of protein, 45% of fat, and 35% of carbohydrate: 26.15 KJ/g total energy percent) for 6 to 8 weeks before the start of the studies. For normal control, same aged rats received a standard rodent diet (30% protein, 60% carbohydrate, and 10% fat, making 12.99 KJ/g total energy, Trouw Nutrition, Cheshire, UK) were used. Before conducting the experiments, fasting blood glucose were measured in high fat fed (HFF) diet rats to separate in individual group. Higher than normal fasting blood glucose levels (5.6 to 7.0 mmol/L) were considered as HFF diet-induced obese rats. The fasting blood glucose of 10 rats were within the normal range which had been excluded from the studies. The groups were divided as follows:

Group 1: Lean control (saline)
Group 2: HFF diet control (saline)
Group 3: HFF diet control + *G. nepalensis* (250 mg/kg)
Group 4: HFF diet control + *G. thomsonii* (250 mg/kg)
Group 5: HFF diet control + *C. infortunatum* (250 mg/kg)
Group 6: HFF diet control + *X. strumarium* (250 mg/kg)
Group 7: HFF diet control + *C. splendens* (250 mg/kg)
Group 8: HFF diet control + Glibenclamide (5 mg/kg)

2.3 Oral Glucose Tolerance

Rats given a high-fat diet were starved for 12 h and administered glucose (18 mmol/kg, body weight (b.w.)) alone (control) or in conjunction with ethanolic extracts (250 mg/kg, b.w.) orally. Blood samples were drawn from the tail vein prior to (0 min) and after (30, 60, 120, and 180 min) treatments. The plasma was separated from the blood by centrifugation at 12,000 rpm for 5 min at 4 °C and the samples were stored at –20 °C for the plasma insulin measurement using Rat Insulin ELISA Kit (Crystal Chem, Elk Grove Village, IL, USA). Ascencia Contour glucose meters (Bayer, Newbury, UK) were used to measure blood glucose levels [24]. Glibenclamide, a sulfonylurea drug, used as a positive control, was dissolved in Dimethyl sulfoxide (0.6% DMSO).

2.4 Feeding Test

HFF rats were used to study the impact of extracts on food consumption. Before the experiment, the rats were fasted for 12 h. Oral administration of saline (5 mL/kg, b.w.), extracts (250 mg/kg, b.w.), or glibenclamide (5 mg/kg, b.w.) was followed by measurement of food intake at 0, 30, 60, 90, 120, and 180 min respectively. Glibenclamide, a standard drug, used as the positive control [25].

2.5 Metabolic Studies

Metabolic studies were performed in HFF Long Evan male rats using metabolic cages to measure food and fluid consumption. The rats underwent 12-h fasting after a 24-h adaptation period. HFF diet control group received saline (5 mL/kg, b.w.), and the treatment groups received plant extracts (250 and 500 mg/kg, b.w.). The food and fluid intake were observed and recorded every 1 h for the first 6 h, then every 2 h for the next 6 h and finally at 24 h [25].

2.6 Gastrointestinal Motility

BaSO4 milk solution was used to assess the gastrointestinal motility. Rats were starved for 20 h and 1 h before administering 10% BaSO4 (W/V of 0.5% Na-CMC) mixture, the treatment groups received extracts (250 mg/kg), bisacodyl (10 mg/kg) and loperamide (5 mg/kg) respectively. After 15 minutes of consuming the BaSO4 milk solution, the animals were killed, and their entire intestines were removed. The distance of BaSO4 travelled was measured and expressed as a fraction of its full length (from the pylorus to ileocecal junction) [25].

Table 1. Preliminary phytochemical screening of five plants (*G. nepalensis*, *G. thomsonii*, C. *infortunatum*, *X. strumarium* and *C. splendens*).

	Alkaloids	Tannins	Saponins	Steroids	Glycoside	Flavonoids	Reducing Sugar
Gynura nepalensis	–	+	+	+	–	+	+
Glochidion thomsonii	+	+	+	–	–	+	+
Clerodendrum infortunatum	–	–	+	–	–	+	–
Xanthium strumarium	–	+	+	–	–	+	–
Clerodendrum splendens	–	+	+	–	–	+	–

(+) = present, (–) = absent.

2.7 Phytochemical Screening

Secondary metabolites such as alkaloids, saponins, steroids, flavonoids, tannins, reducing sugar, and glycosides were identified using previously described techniques [26]. Alkaloids were tested by acidifying 2 mL of the extracts with hydrochloric acid (HCl), to which 1 mL Dragendroff's reagent was added, and the appearance of a red color showed the presence of alkaloids. To test for tannins, a few drops of 10% lead acetate were added to 2 mL of the extracts and the formation of white sediment confirmed the presence of tannins. Testing for the presence of flavonoids involved heating a mixture of 4 mL of the extracts and 1.5 mL methanol; the appearance of a pink color upon the addition of magnesium and a few drops of HCl suggested the presence of flavonoids. To test for saponins, 1 mL of the extracts was mixed with 9 mL distilled water which produced a stable foam suggesting the presence of saponins. To check the presence of steroids, 2 mL of the extracts were mixed with 10 mL chloroform, 1 mL acetic anhydride, and 2 mL sulphuric acid; the formation of a bluish-green color indicated the presence of steroids. Glycosides were tested by combining 1 mL of the extracts with a few drops of glacial acetic acid, ferric chloride, and concentrated sulphuric acid; visualization of bluish-green color suggested the presence of glycosides. Reducing sugars were tested by combining 1 mL of the extracts, 1 mL of distilled water, and a few drops of Fehling's reagent. The mixture was heated, and visualization of a red-brick color indicated the presence of reducing sugars.

2.8 Statistical Analysis

To analyse and interpret the data, Graph Pad prism 5 (San Diego, CA, USA) was used. Data analysis was done using an unpaired Student's *t*-test (nonparametric, with two-tailed *p* values) and a one-way ANOVA with Bonferroni post hoc testing, and the values were represented as Mean $\pm$ SEM with a hypothetical significance level of $p < 0.05$.

3. Results

3.1 Oral Glucose Tolerance and EEMPs

Oral administration of G. *nepalensis, G. thomsonii, C. infortunatum* and *C. splendens* (250 mg/kg), when given in combination with glucose (18 mmoL/kg body weight) significantly ameliorated glucose tolerance in HFF rats at 30 and 60 min ($p < 0.05$–0.01; Fig. 1A–C,E) as compared to glucose alone. *G. nepalensis* and *C. splendens* (250 mg/kg) also improved blood sugar levels at 120 min compared to HFF rats ($p < 0.05$; Fig. 1A,E), whereas *X. strumarium* (250 mg/kg) improved glucose tolerance only at 30 min ($p < 0.01$; Fig. 1D). Glibenclamide (5 mg/kg) also enhanced ($p < 0.05$–0.01; Fig. 1A–E) glucose tolerance in HFF rats. All the plants extract improved plasma insulin levels at 30 min ($p < 0.05$; Fig. 2A–E). *G. nepalensis* and *C. infortunatum* also improved plasma insulin level at 60 min ($p < 0.05$; Fig. 2A,C). A positive control, glibenclamide (5 mg/kg) increased ($p < 0.05$–0.001; Fig. 2A–E) plasma insulin level in HFF rats at 30 and 60 min respectively.

3.2 Feeding Test and EEMPs

G. nepalensis (250 mg/kg), caused a significant decrease in food intake at 120 and 180 min ($p < 0.001$; Fig. 3A) whereas *G. thomsonii*, *C. infortunatum* and *C. splendens* (250 mg/kg) were constantly significant ($p < 0.05$–0.001; Fig. 3B,C,E). *X. strumarium* (250 mg/kg) reduced food consumption at 60, 90, 120 and 180 min ($p < 0.05$–0.01; Fig. 3D) respectively. Glibenclamide (5 mg/kg) also improved ($p < 0.05$–0.01; Fig. 3A–E) food intake in HFF rats.

3.3 Metabolic Studies and EEMPs

Although ethanol extracts of *G. nepalensis, G. thomsonii, C. infortunatum, X. strumarium* and *C. splendens* (250 and 500 mg/kg, b.w.) decreased food and fluid intake in HFF rats, over the period of 36 h, was not significant in comparison to the control (Figs. 4A–E,5A–E). However, extracts, at 500 mg/kg, b.w. was more effective than at 250 mg/kg, b.w. (Figs. 4A–E,5A–E).

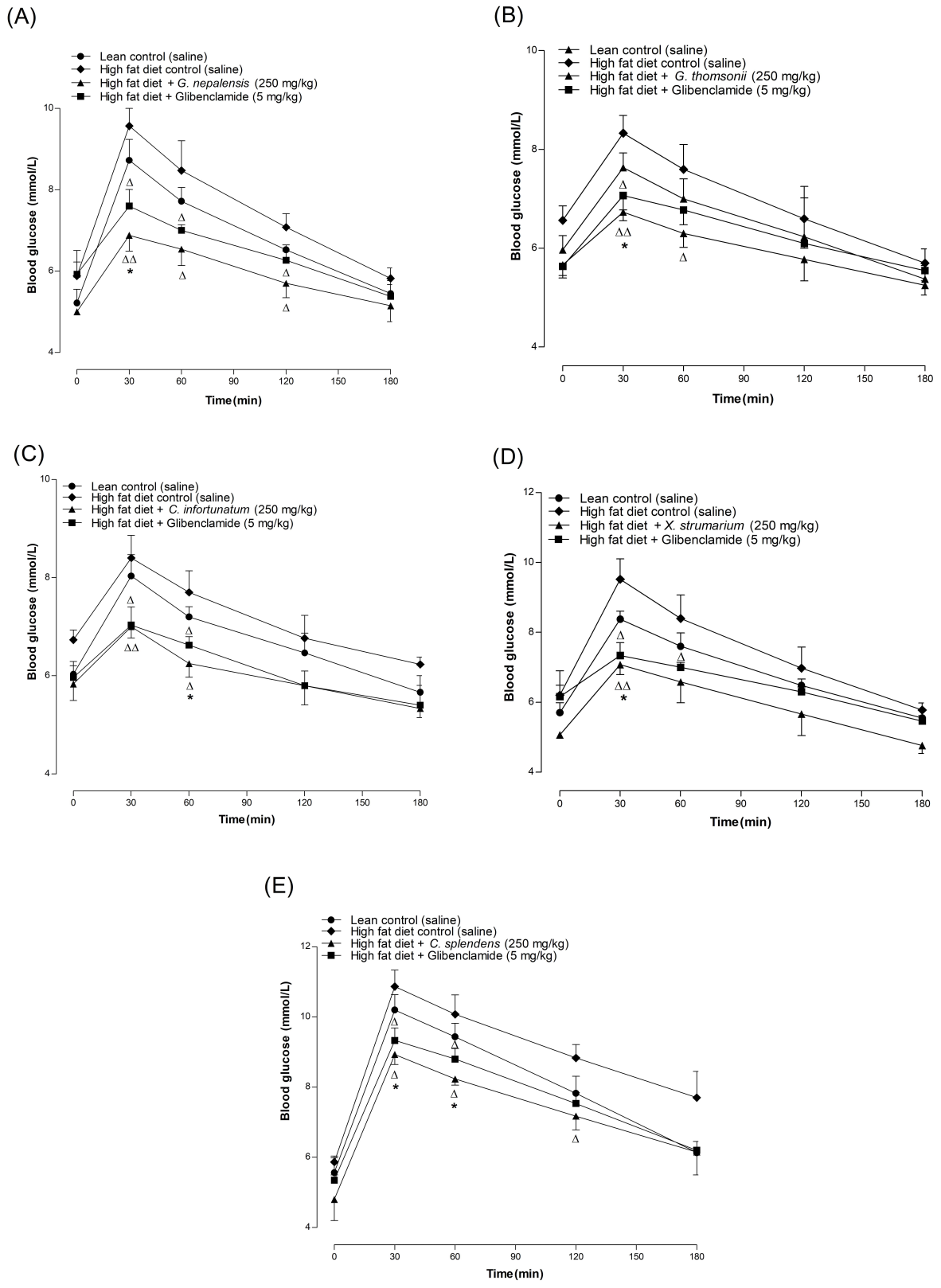

Fig. 1. Effects of ethanol extracts of (A) *G. nepalensis*, (B) *G. thomsonii*, (C) *C. infortunatum*, (D) *X. strumarium* and (E) *C. splendens* on oral glucose tolerance in HFF rats. Blood glucose was monitored in overnight fasted rats before and after oral gavage of glucose (2.5 gm/kg, body weight, control), with or without a plant extracts (250 mg/kg, body weight) or glibenclamide (5 mg/kg). Values n = 6 are mean ± SEM. * $p < 0.05$ compared to control (saline). Δ, ΔΔ $p < 0.05$–0.01 compared to high-fat-fed diet control rats. Glibenclamide was used as a positive control.

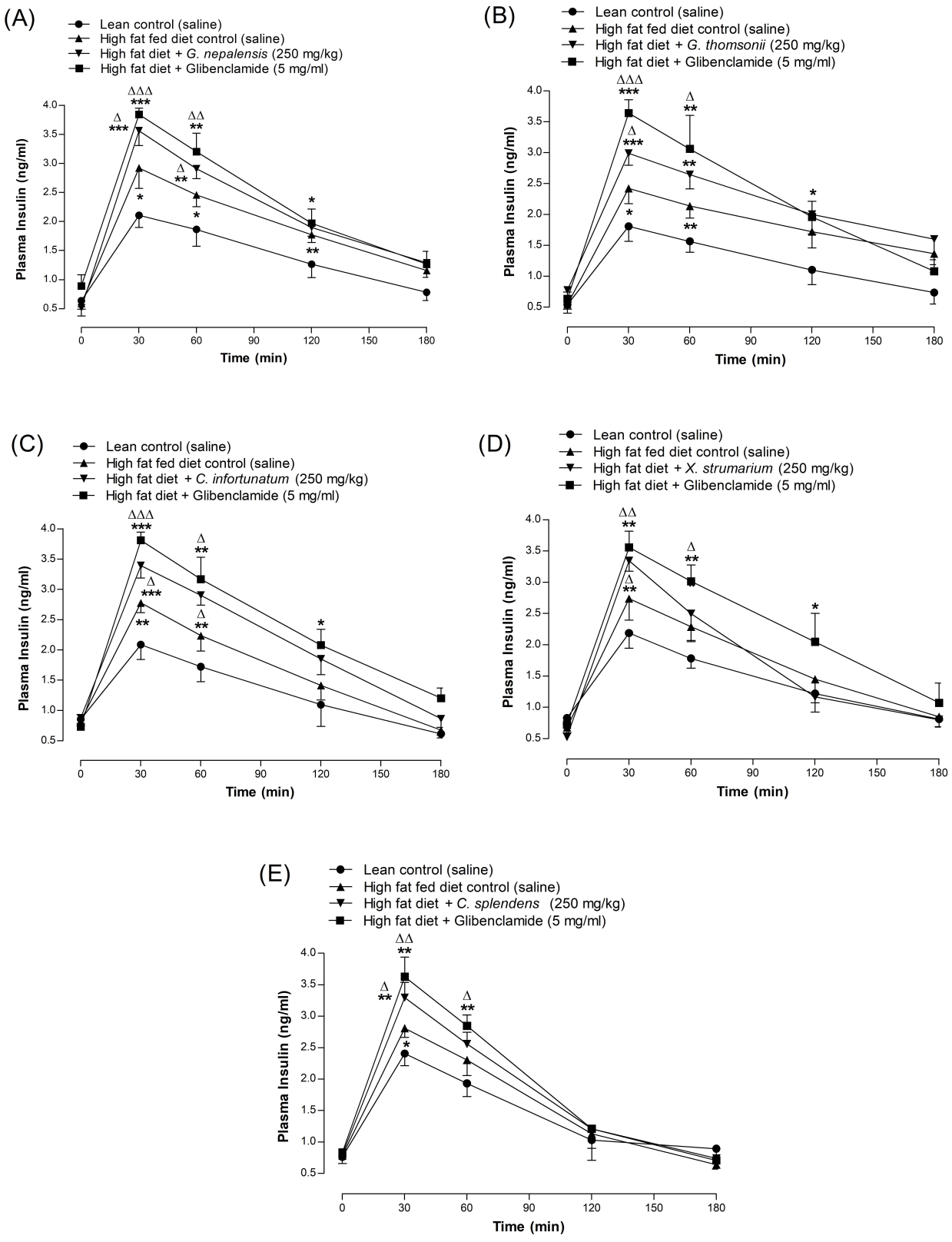

Fig. 2. Effects of ethanol extracts of (A) *G. nepalensis*, (B) *G. thomsonii*, (C) *C. infortunatum*, (D) *X. strumarium* and (E) *C. splendens* on plasma insulin levels in HFF rats. Plasma insulin was monitored in overnight fasted rats before and after oral gavage of glucose (2.5 gm/5 mL/kg, body weight, control), with or without a plant extracts (250 mg/kg, body weight) or glibenclamide (5 mg/kg). Values n = 6 are mean ± SEM. *, **,*** $p < 0.05$–0.001 compared to control (saline). Δ, ΔΔ, ΔΔΔ $p < 0.05$–0.001 compared to high-fat-fed diet control rats. Glibenclamide was used as a positive control.

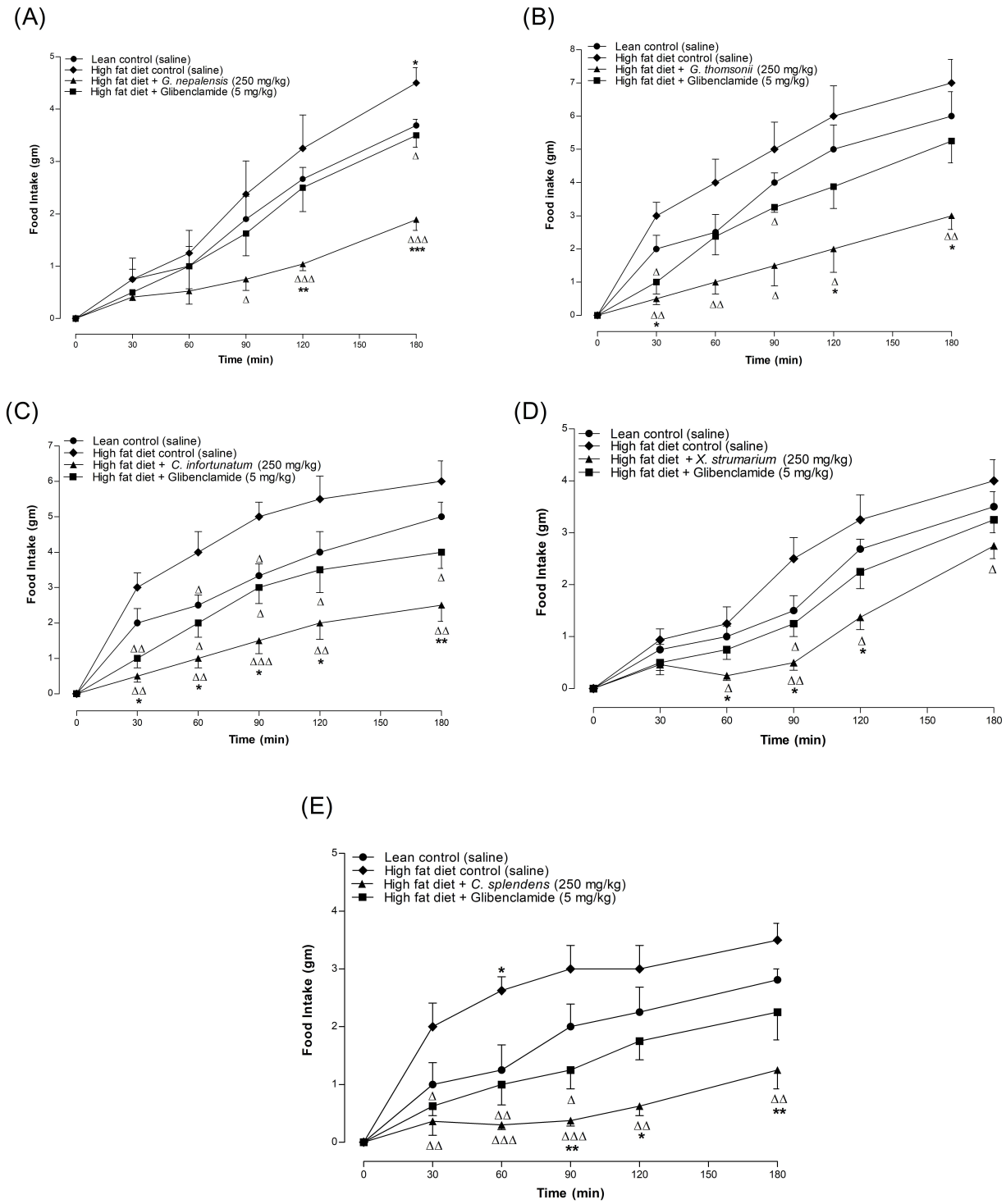

Fig. 3. Effects of ethanol extracts of (A) *G. nepalensis*, (B) *G. thomsonii*, (C) *C. infortunatum*, (D) *X. strumarium* and (E) *C. splendens* on food intake in HFF rats. Food intake was assessed in 12 h fasted rats with or without oral administration of plant extracts (250 mg/kg, body weight) or glibenclamide (5 mg/kg). Values n = 6 are mean ± SEM. *, **, *** $p < 0.05$–0.001 compared to control (saline). Δ, ΔΔ, ΔΔΔ $p < 0.05$–0.001 compared to high-fat-fed diet control rats. As a positive control, glibenclamide was used.

3.4 Gastrointestinal Motility and EEMPs

The ethanol extract of plants, *G. nepalensis*, *G. thomsonii*, *C. infortunatum*, *X. strumarium* and *C. splendens* (250 mg/kg) markedly enhanced gut motility ($p < 0.05$–0.001; Fig. 6) in HFF rats. Bisacodyl, a laxative (10 mg/kg) improved gut motility ($p < 0.01$–0.001; Fig. 6), whereas loperamide (5 mg/kg), an antidiarrheal drug, lowered gut motility ($p < 0.05$; Fig. 6) as compared to HFF diet control rats.

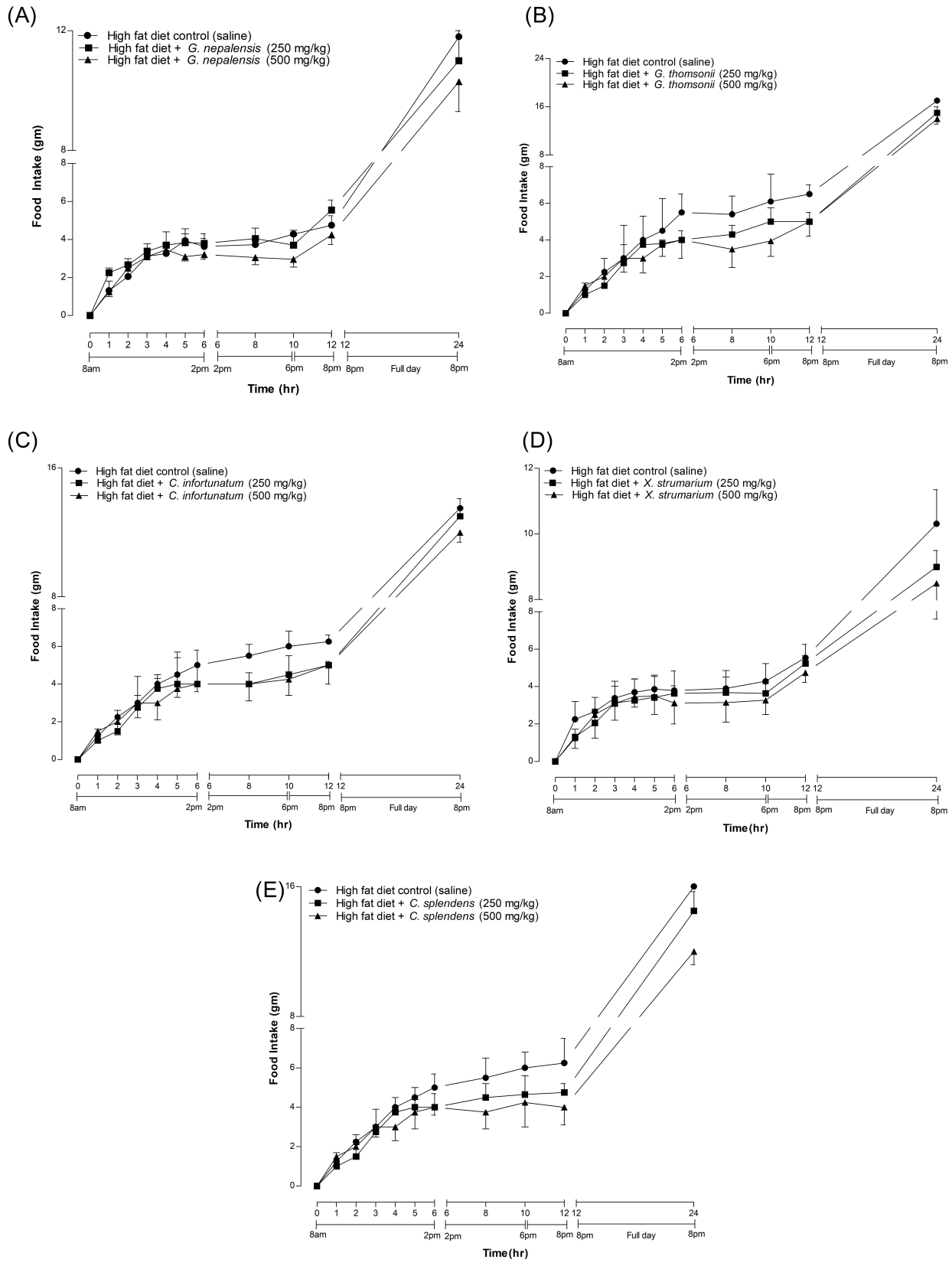

Fig. 4. Effects of ethanol extracts of (A) *G. nepalensis*, (B) *G. thomsonii*, (C) *C. infortunatum*, (D) *X. strumarium* and (E) *C. splendens* on food intake after 36 h of metabolic study. Food intake was measured at 1, 2, 3, 4, 5, 6, 8, 10, 12 & 24 h interval under metabolic cage along with or without either receiving a plant extracts (250 & 500 mg/kg, body weight). Values n = 6 are mean ± SEM. HFF rats alone were used as control.

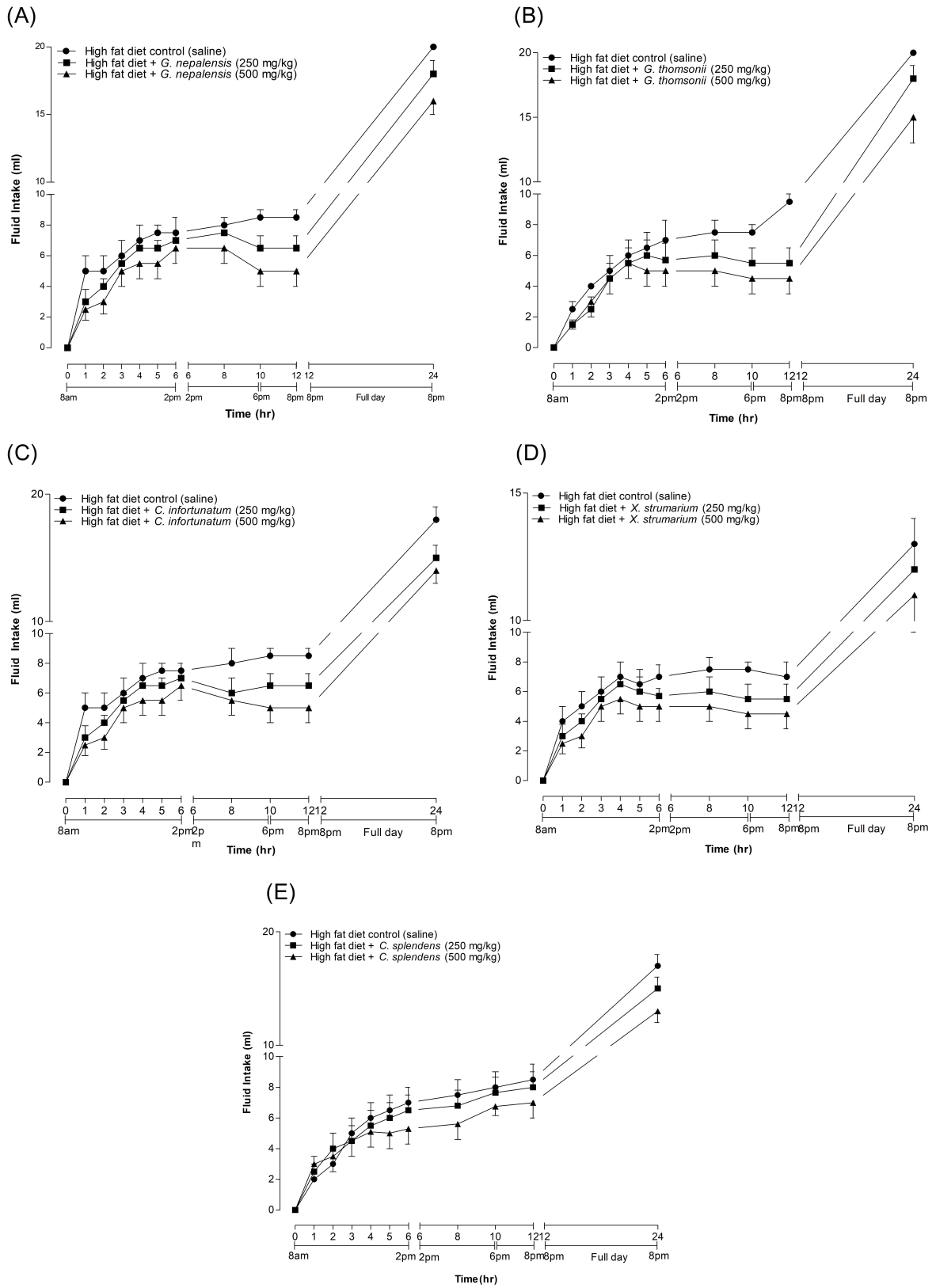

Fig. 5. Effects of ethanol extracts of (A) *G. nepalensis*, (B) *G. thomsonii*, (C) *C. infortunatum*, (D) *X. strumarium* and (E) *C. splendens* on fluid intake after 36 h of metabolic study. Fluid consumption was assessed at 1, 2, 3, 4, 5, 6, 8, 10, 12 and 24 h intervals using metabolic cage along with or without a plant extracts (250 & 500 mg/kg, body weight). Values n = 6 are mean ± SEM. HFF rats alone were used as control.

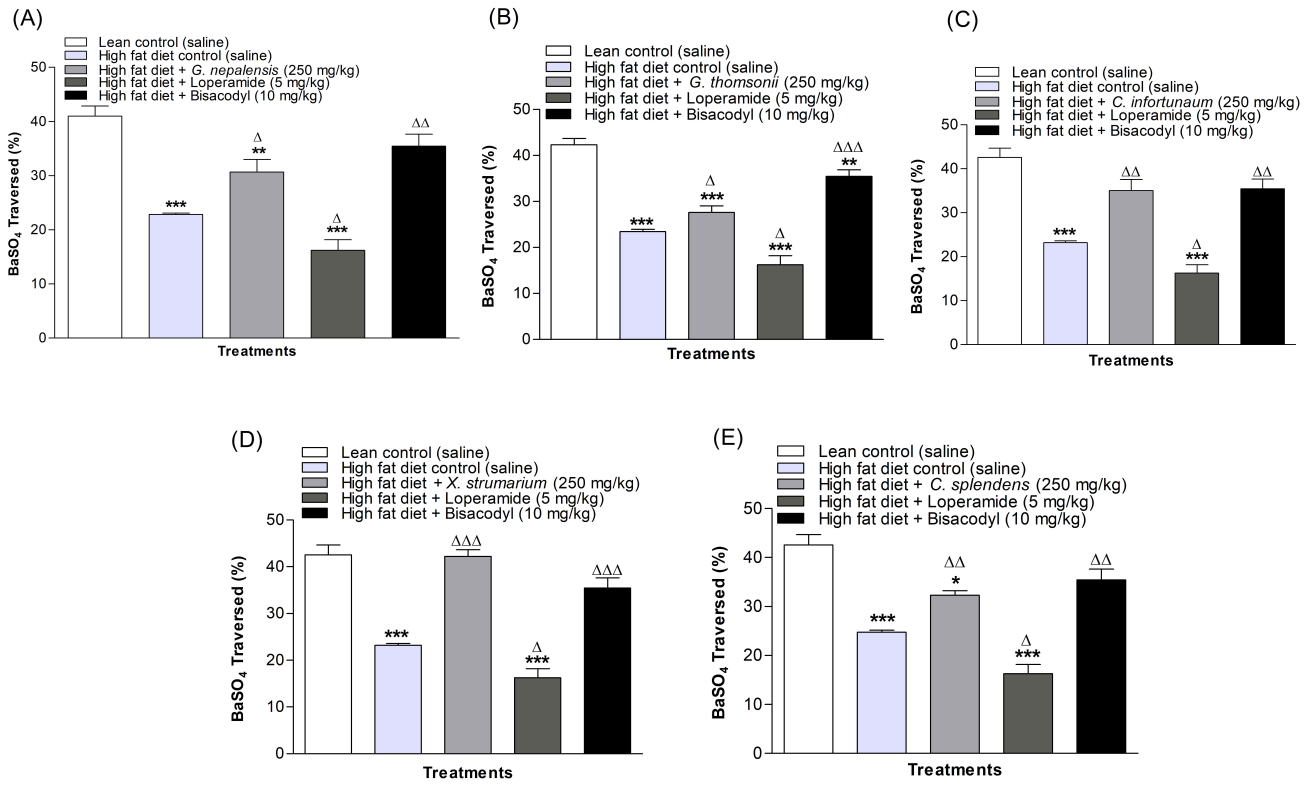

Fig. 6. Effects of ethanol extracts of (A) *G. nepalensis*, (B) *G. thomsonii*, (C) *C. infortunatum*, (D) *X. strumarium* and (E) *C. splendens* on gastrointestinal motility. BaSO4 milk solution was used to evaluate gastrointestinal motility by measuring BaSO4 travel length in 20 h fasted rats after oral gavage of BaSO4 solution with or without a plant extracts (250 mg/kg, body weight), loperamide (5 mg/kg) or bisacodyl (10 mg/kg). Values n = 6 are mean ± SEM. *, **, *** $p < 0.05$–0.001 compared to control (saline). Δ, ΔΔ, ΔΔΔ $p < 0.05$–0.001 compared to high-fat-fed diet control rats. Loperamide and bisacodyl were used as positive controls.

3.5 Phytochemical Screening and EEMPs

Saponins and flavonoids were detected in all five plants in phytochemical screening of crude extract, as well as tannins in *G. nepalensis*, *G. thomsonii*, *C. splendens*, and *X. strum*arium (Table 1). Reducing sugar was found in *G. nepalensis* and *G. thomsonii* and alkaloids were only present in *G. thomsonii* (Table 1). Additionally, *G. nepalensis* also contained steroids (Table 1).

4. Discussion

Diabetes, one of the most prominent and severe metabolic disorders, has greatly affected many individuals all over the world [27]. Obesity, commonly characterized to be an excess of body fat, is a major risk factor for T2DM. In people who are obese, adipose tissues produce nonesterified fatty acids (NEFAs), which may result in insulin resistance and β-cell dysfunction, and this may ultimately lead to T2DM [28]. Obesity may also potentially raise the risk of cardiovascular disease (CVD) in T2DM patients by deteriorating hypertension and dyslipidaemia [29]. Therefore, we chose HFF rats to conduct *in vivo* studies to provide an idea on how specific medicinal plants may interact with T2DM and its complications. At this preliminary study, the anti-diabetic potential of ethanol extract of five traditional medicinal herbs *G. nepalensis*, *G. thomsonii*, *C. splendens*, *C. infortunatum*, and *X. strumarium* were observed.

In acute *in vivo* studies, the ethanol extracts of *G. nepalensis*, *G. thomsonii*, *C. splendens*, *C. infortunatum* and *X. strumarium* were found to substantially ameliorate glucose tolerance and plasma insulin level in HFF rats, suggesting that all five plants have glucose lowering properties. Recent reports have demonstrated that *C. infortunatum* improves glucose tolerance and reduces fasting blood glucose in streptozotocin induced diabetic rats [30]. Previous research on *G. nepalensis* and *X. strumarium* showed that they possess α-glucosidase inhibitory activities, and they can also reduce oxidative stress damage via free radical scavenging [31,32]. The glucose lowering properties of *G. thomsonii* and *C. splendens* may be due to the presence of phytochemicals such as diosgenin, gymnemic acid, epigallocatechin gallate, catechin, proanthocyanidin and ellagic acid, are known to improve glucose homeostasis by reducing blood glucose levels, inhibiting glucose absorption, improving pancreatic β-cell function, and decreasing oxidative stress damage [33–35].

Additional *in vivo* studies on HFF rats comprised feeding tests and metabolic studies to investigate the effect of plant extracts on food and fluid consumption. The plant ex-

tracts reduced both fluid and food consumption but was not significant as compared to HFF control. Plant extracts with high dose (500 mg/kg, b.w.) shown more efficacy in decreasing these parameters, particularly at night (between 6 to 8 PM). Rats are known to be more active during nighttime and the blood sugar levels in diabetic rats tend to be at peak at night [36]. Thus, reduction in food intake caused by medicinal plant extracts may be responsible for maintaining healthy blood glucose levels.

Gastrointestinal motility was observed using BaSO4 milk solution. The present studies showed that plant extracts promote gut motility, indicating that the plants may shorten the time available for the digestion and absorption of carbohydrates in the gut, leading to decreased glucose absorption and plasma sugar levels [37]. The presence of saponins in these plants may contribute to improve gut motility, as saponins are known to inhibit disaccharidase activity and intestinal glucose absorption in streptozotocin induced rats [38].

Phytochemical screening of the five plant extracts confirmed the presence of flavonoids and saponins, which is consistent with previous studies on these plants [39–43]. Flavonoids, such as kaempferol, rutin and quercetin, have previously been demonstrated to have glucose lowering, insulin secreting, and β-cell protecting properties in different animal models, including streptozotocin and alloxan induced rats, and HFF rats [44–46]. Recent studies on saponins have revealed that these phytochemicals can enhance insulin release and inhibits absorption of carbohydrates by mediating the AMP-activated protein kinase (AMPK) pathway [47]. Tannins were found in four of these plants (*G. nepalensis*, *G. thomsonii*, *C. splendens*, and *X. strumarium*) is consistent with prior findings [46–49]. Recent reports indicated that tannins enhance glucose uptake in 3T3L1 adipocyte cells and reduce oxidative stress via the phosphatidylinositol (PI3) and mitogen-activated protein kinases (MAPK) pathways [48,49]. Alkaloids found in *G. thomsonii*, are also known to be efficacious α-glucosidase inhibitors [50,51]. The anti-diabetic properties of these five plants could be attributed due to the presence of these phytochemicals. However, further studies, including *in vitro* cell line experiments, and chronic *in vivo* studies are warranted to fully comprehend the antidiabetic and insulinotropic properties of these plants.

5. Conclusions

Traditional medicinal plants, *G. nepalensis*, *G. thomsonii*, *C. splendens*, *C. infortunatum* and *X. strumarium*, showed improvement in glucose tolerance, plasma insulin level and gastrointestinal motility in HFF rats, suggesting that the extracts may improve diabetes and its complexities by lowering blood sugar levels and inhibiting carbohydrate digestion and absorption in the gut. In addition, the plants were also observed to reduce consumption of food and fluid in HFF rats. The anti-hyperglycaemic properties of these plants may be attributed to the presence of phytochemicals such as flavonoids and saponins. Our findings support the use of these plants as an ethnomedicine for the treatment of T2DM. Additional in-depth research, such as the purification and identification of active components from these plants, could aid in the development of anti-diabetic therapy for T2DM in humans.

Availability of Data and Materials

The information is not available to the public as a result of certain limitations. The corresponding author, however, is willing to provide the information acquired from this study upon request.

Author Contributions

JMAH and PA were equally responsible for the conception and design of the study as well as the supervision of the study; PA, NN, FTT and AT conducted the experiments, analyzed the data, evaluated the results, created the figures, and PA drafted the paper, while PA and JMAH edited the revised manuscript. All authors have participated sufficiently in the work to take public responsibility for appropriate portions of the content and agreed to be accountable for all aspects of the work in ensuring that questions related to its accuracy or integrity. The final manuscript has been read and approved by all authors.

Ethics Approval and Consent to Participate

Independent University, Bangladesh (IUB), Institutional Review Board (IRB) approved protocols on 19th December 2019 for experiments to be performed using animals and the experiments were performed in line with the Animal Welfare Act 2019 of Bangladesh. It was ensured that no animals will be injured over the course of this study.

Acknowledgment

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Conflict of Interest

The authors declare that there is no conflict of interest.

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