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Formulation and evaluation of capsules and suppositories containing Gongrenema *Latifolium*

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ABSTRACT

Introduction: Earlier studies have been done on *Gongronema latifolium* (GL) for anti-diabetic effect using animal model. However, the extracts have bitter taste. This study focused on the formulation and evaluation of solid dosage forms namely capsules and suppositories.

Method: Purified GL were used in formulation of capsules and suppositories.The capsules were evaluated for various physico-chemical properties such as weight uniformity, disintegration time and dissolution test. Physical properties of suppositories were also assessed. Investigation on anti-diabetic effects of capsules and suppositories of purified *Gongronema latifolium* on Streptozotocin-induced diabetic rats was carried out.

Results: Weight uniformity tests of the capsules showed percentage deviation of less than 10%. Disintegration of the capsules was within 2 min. Drug release profile showed that peak drug release occurred at 12 min. Physical properties of suppositories were satisfactory. The antidiabetic effect of capsules was 52± 11.82 %. C-peptide (pM) of GL purified capsules was 115.76 ±1.54 respectively. Blood glucose of diabetic guinea pig was not significantly reduced when varying concentrations of GL suppositories were administered.

Conclusion: Capsules and suppositories passed compendia requirements, while purified *Gongronema latifolium* capsules demonstrated significant anti-diabetic activities unlike the suppositories. Suppository is not an ideal dosage form for *Gongronema latifolium* anti-diabetic herb.

1. Introduction

Gongronema latifolium (*Asclepiadaceae*) is an edible rainforest plant, native to the Southeastern part of Nigeria and widely used in folk medicine as a spice and vegetable. The plant is also used by herbalist in Nigeria folk medicine for the treatment of diabetes mellitus[1,2], anti-diarrhea and anti-tussive. The herbal extract is prepared by grinding a known quantity of the fresh leaves of the plant and dispersing in an appropriate volume of water. The mixture is then filtered and administered orally to patients in a chosen dose, two times daily[2]. Earlier studies on the crude leaf extract of the plant reported hypoglycemic, hypolipidemic, anti-microbial, anti-cancer, anti-inflammatory, and anti-oxidative effects in diabetic rats [3,4].

Diabetes Mellitus (DM) has now become an epidemic with a worldwide incidence of 5 % of the world's population. In 2008, it killed more than acquired immune deficiency syndrome (AIDS) [5]. The World Health Organization (WHO) estimated that about 350 million people are living with diabetes [6]. It was also predicted that global diabetes prevalence would increase by 50 % in 2030 [6]. Diabetes

mellitus is associated with long term complications such as retinopathy, neuropathy and angiopathy. The use of orthodox drugs in the management of DM has not improved the situation. It has also been reported by the World Health Organization (WHO) that about 80 % of the world's population use herbal medicine[7]. Liquid *G latifolium* herbal extracts characteristically have a bitter taste. The purpose of this work was to formulate solid dosage forms of GL namely capsules and suppositories. Capsules mask the bitter taste and encourage patient to adhere to therapy. Suppository is alternatively use when patient is unable to tolerate orally administered medication.

2. Materials and Method

2.1 Materials

Methanol, Chloroform, Hexane (Sigma Aldrich, Germany), lactose (Santa Cruz USA), Gelatine, Silica gel (Qualikems, China).Metformin HCl (Sigma Aldrich, Germany), Glibenclamide (Santa Cruz, USA), Streptozotocin mixed anomers SO130 (Sigma Aldrich Co, St Lious, USA)

2.1.2 Animal

Inbred albino Wistar male rats (weighing 100 -140 g) were bred in the Laboratory Animal Unit of Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University, Awka Nigeria, and were used for some of the experiments. The environmental temperature varied between 18 and 30 ^{o}C and the lighting period was between 15 ± 2h daily. The rats were given clean drinking water and fed with standard commercial pelletized growers feed (Vital Feed, Nigeria).

2.2 Methods

2.2.1 Aqueous extraction of GL leaves

The leaves of *Gongronema latifolium*(GL) were collected from Enugu in Enugu North Local Government Area, Enugu State, Nigeria. The fresh leaves were washed and air dried in a room. They were pulverized into a fine powder using a blender, sealed in polythene bags and stored at 4 ^{o}C in refrigerator. A 200 g powdered leaf of GL was extracted by immersing it in distilled water for 48 h. They were shaken at regular intervals of 2 h. The extracts were filtered with muslin cloth and then with filter paper (No 1 Whatmann). The extracts were freeze-dried and stored in the freezer prior to the preliminary screening.

2.2.2 Preparation of methanol extract (ME) of GL

A 200 g quantity of GL was extracted by cold maceration. The GL was immersed in aqueous methanol (80 %) for 48 h and shaken every 2 h. It was filtered and dried in a rotary evaporator to obtain the methanol extract (ME).

2.2.3 Isolation of anti-diabetic active fractions of MEGL leaf

The ME was further fractionated using chromatographic techniques, with the following solvents in the order of increasing polarity viz n- hexane, chloroform and methanol.

2.3 Preliminary screening for anti-diabetic activity of GL aqueous extract

The aqueous extracts and isolated fractions of the GL were subjected to phytochemical tests for the presence of phytochemical constituents using the method described by Trease and Evans [8].

Thirty healthy male albino rats were randomly selected and housed in five groups (1-5).The animals were fed on standard growers pellet diet (Vital feed and Nigeria) and water *ad libitum*. The animals were fasted for 24 h before induction of diabetes (2-5groups) by intra-peritoneal (IP) injection of a single dose of Streptozotocin (STZ) (65 mg/kg body weight). The weighed sample was dissolved in distilled water and injected immediately to avoid degradation. Diabetes was confirmed after 48 h in rats that showed fasting blood glucose (FBG) levels of > 240 mg/dL. The animals in groups 1 and 5 were designated normal and diabetic controls respectively and received 2mL/kg of normal saline. Animals in group 2, received GL (400 mg/kg) while groups 3 and 4 received standard drug Glibenclamide (2 mg/kg) and Metformin (500 mg/kg) respectively. The blood glucose of all the rats was measured at predetermined times (0, 1, 2, 4, 8 h) using Accu-check ® Advantage (Roche). The percentage glucose reduction was calculated.

2.4 Fourier transform infrared spectroscopy (FTIR) analysis

Fourier transform infrared spectroscopy measurement of the drug, individual excipient and drug and excipient mixture were obtained on Shimadzu FTIR. The mixture of powder sample and potassium bromide were prepared in the form of potassium bromide pellets. The spectrum obtained by scanning over the wave- number range of 4000-400 cm^{-1} at ambient temperature. The spectral data of these scans were used to determine the compatibility of drugs and excipients.

2.5 Determination of some physical properties of powder blend of extracts and excipients

Some physical properties of powder blends of extracts and excipients for preparing extract capsules were determined by calculating the bulk density, tapped density, Hausner ratio, Carr's index and angle of repose.

2.6 Formulation of dosage forms

2.6.1 Formulation of capsule dosage form

The pre-formulation formula for aqueous leaf extracts and semi-purified of GL was developed. The formula 2 was best for GL aqueous extract capsules. Lactose and talc were compatible with GL. Lactose was used as diluents while talc was used as lubricant. The powder composed of drugs and excipients was mixed in a glass mortar for 20 min. The powder blend was filled into No 2 hard gelatin capsules (260 mg) using a semi-automatic capsule-filling machine (Capsul CN, China) as reported by USP[9].

Table 1: Pre-formulation formula for preparing GL aqueous leaf extract capsules

Drugs/Excipients	Formula 1(g)	Formula 2 (g)	Formula 3(g)
GLALE	1.6	1.6	1.6
Lactose	1.8	1.8	1.2
Talc	0.6	1.2	0.6

Key: GLALE = *Gongronema latifolium* aqueous leaf extract

2.6.2 Formulation of suppositories

Glycerinated gelatine was used as the base for preparation of suppository using a developed formula. The GL semi-purified suppositories were prepared using plastic mold as reported in USP 2009[9]. Storage of suppositories was done in refrigerator (1.7 – 3.3°C). Glycerinated suppositories were protected from moisture by storing in a well closed container.

2.7 Evaluation of capsule dosage forms

Physical examination of all the anti-diabetic capsules was carried out visually. Weight uniformity test was performed for 20 capsules per extract. The percent weight deviation was calculated as described by Talman[10]. *In vitro* disintegration time was determined for GL capsules using the disintegration test apparatus (Logan Instrument, Germany). Each of the 6 tubes was filled with a capsule and a disc. The distilled water was maintained at a temperature of 37 ± 0.5°C and the time taken for complete disintegration of the capsules was noted.

2.7.1 Determination of maximum wavelength and *In-vitro* release studies

A 100 mg quantity of GL extract was added into a 100 mL volumetric flask, dissolved in 60 ml water and sonicated for 10 min. The volume was made up to the mark and filtered through Whatmann filter paper No 1. After appropriate dilution with water, the maximum wavelength (λ max) was determined by scanning using ultra violet (UV) spectrophotometer (PerkinElmer Lambda 35, Germany). Thereafter, different concentrations were used to prepare a Beer's plot of GL using the appropriately determined λ max. Each capsule was subjected to *in-vitro* dissolution studies in 900 mL of distilled water, pH 7 for 2 h using a USP XXIII dissolution apparatus (Bender and Hoban per Laborfachach, Germany) at 50 rpm maintained at 37± 0.5°C. A 5 mL quantity of the dissolution medium was withdrawn every 10 min and filtered through Whatman filter paper 2, diluted ten folds, and analyzed using UV-visible double beam spectrophotometer at 274 nm which was obtained as maximum wavelength from the UV Spec scan. Equal amounts of fresh dissolution medium were replaced immediately after withdrawing 5 mL.

2.7.2 Determination of dose-related anti-diabetic response of semi-purified GL capsules

Forty-eight rats of male sex weighing between 120-150 g were randomly divided into 8 groups (1 –8) of six rats per group and fasted for 14 h. Each rat in each group was intraperitoneally (IP) injected with STZ (65 mg /kg body weight) in distilled water. The animals were fed with standard pellet growers feed diet (Vital Feed, Nigeria) and water *ad libitum* for 48 h. Diabetes was confirmed after 48 h in rats that showed fasting blood glucose (FBG) levels of > 240 mg/dL. The control group (1) and diabetic group (8) each received 2 ml/kg of normal saline. The semi purified GL capsules were administered orally at doses of 400, 600, 800, 1200 mg/kg to groups 2-5 using an improvised method. Then standard drugs Glibenclamide (2 mg/kg) and Metformin (500 mg/kg) were administered to groups 6 and 7 respectively. Blood samples were collected from the tail vein after overnight fast at the intervals of 0, 2, 4 and 8 h. The blood glucose was measured using Accu-check glucometer (Roche, USA) and percentage glucose reduction was calculated.

2.7.3 Capsule Delivery

A 1 mL syringe was used as improvised device for delivering the capsules into the stomach of the rats. The tip where the needle is normally inserted was cut off. The capsule was placed at the cut end of the syringe. The rat was restrained by holding the neck muscle. The device with the inserted capsule was placed inside the mouth at the dorsal part of the tongue (roof of the tongue), pushing the plunger delivered the capsule inside the stomach through the esophagus. Water was then given to the animal.

Figure 1: The Improvised Devise for oral delivery of capsule

2.7.4 C-peptide determination in diabetic animals treated with G latifolium capsules

The plasma was separated from blood samples of treated diabetic animals immediately after collection. Then quantitative determination of C-peptides was carried out using standard Elisa kit obtained from ALPCO, USA.

2.8 Dose-related anti-diabetic response of GL leaf extract suppository on STZ- induced diabetic rat

Physical observation of the GL suppository extract was done visually. Properties observed were colour, clarity, surface texture, appearance and feel. Twenty- four male rats weighing between 120–150 g were randomly divided into 4 groups (1-4) of six rats per group and fasted for 14 h. Each rat in each group was intraperitoneally (IP) injected with determined volume of STZ (65 mg /kg body weight) to induce diabetes. The animals were fed on standard pellet diet (growers feed, Vital Feed, Nigeria) and water *ad libitum* for 48 h. Diabetes was confirmed after 48 h in rats that showed fasting blood glucose (FBG) levels of > 240 mg/dL. The semi-purified GL suppository was administered rectally at doses of 400, 600, 800, mg/kg body weight for groups 1-3. Then standard Glibenclamide (2 mg/kg) was administered rectally to group 4. Blood glucose of the treated rats was measured at 0, 1, 2, 4 and 8 h using Acccu-check ® Advantage glucometer and percentage blood glucose was calculated.

2.9 Statistical analyses

The results generated from the various determinations were expressed as mean ± standard deviation. The differences between the data sets were determined using one way analysis of variance (ANOVA). Variant means were separated post-hoc using Turkey's HSD. ρ values less than 0.05 was considered significant.

3. Results

Herbal plants have been shown to possess anti-diabetic activity. In this study, solid dosage forms of GL namely capsule, and suppositories were formulated and evaluated. Chromatographic separation of GL methanol extract produced methanol fraction as the highest yield (90%).The phytochemical tests of aqueous extract and methanol fraction of GL are shown in Table 2. The result of phytochemical analysis of aqueous extract showed that all the extracts contain saponins, flavonoids, tannins, and alkaloids.

Table 2: Phytochemical results of methanol fraction and aqueous extract of GL

Constituents	Methanol Fraction	Aqueous Extract
Flavonoids	++++	++
Saponins	++++++	++++
Alkaloids	++	++
Tannins	+	+
Starch	++	++
Glycoside	++	++
Carbohydrates	++	++

Key: + = small ++ =moderate +++ = high ++++ = very high** (quantified subjectively)

The percentage blood glucose reduction of GL aqueous leaf extract is 53 %. The results of the anti-diabetic screening of fractions of GL methanol extract (GLME) are presented in Figure 2. Methanol fractions had the highest anti-diabetic activity (68 %) at the dose of 400 mg/kg body weight. The N- hexane fraction of GLME had the lowest anti-diabetic activity (24 %) at the same dose.

3.1 Fourier transforms infrared spectroscopy (FTIR)

The FTIR analysis is a nondestructive and non disruptive method that provides a means to probe the molecular level interactions between polymers. The FTIR plots are showed in Figures 3A-D.The GL methanol fraction and aqueous extract was compatible with lactose. The aqueous leaf extract of GL was compatible with talc except for slight interaction at wave number of 3825cm^{-1}. The GL methanol fraction was compatible with gelatin.

3.2 Physical properties of powder mix of extract and excipients

The tapped density of GLPE was 0.16 while .bulk density was 0.227. The angle of repose for GLALE was 27.33^{O}. This Carr's index and Hausner ratio can be determined on small quantities of powder. The powder blends of GLALE and GLPE had Hausner ratio > 1.25 and Carr's index of > 20 %.

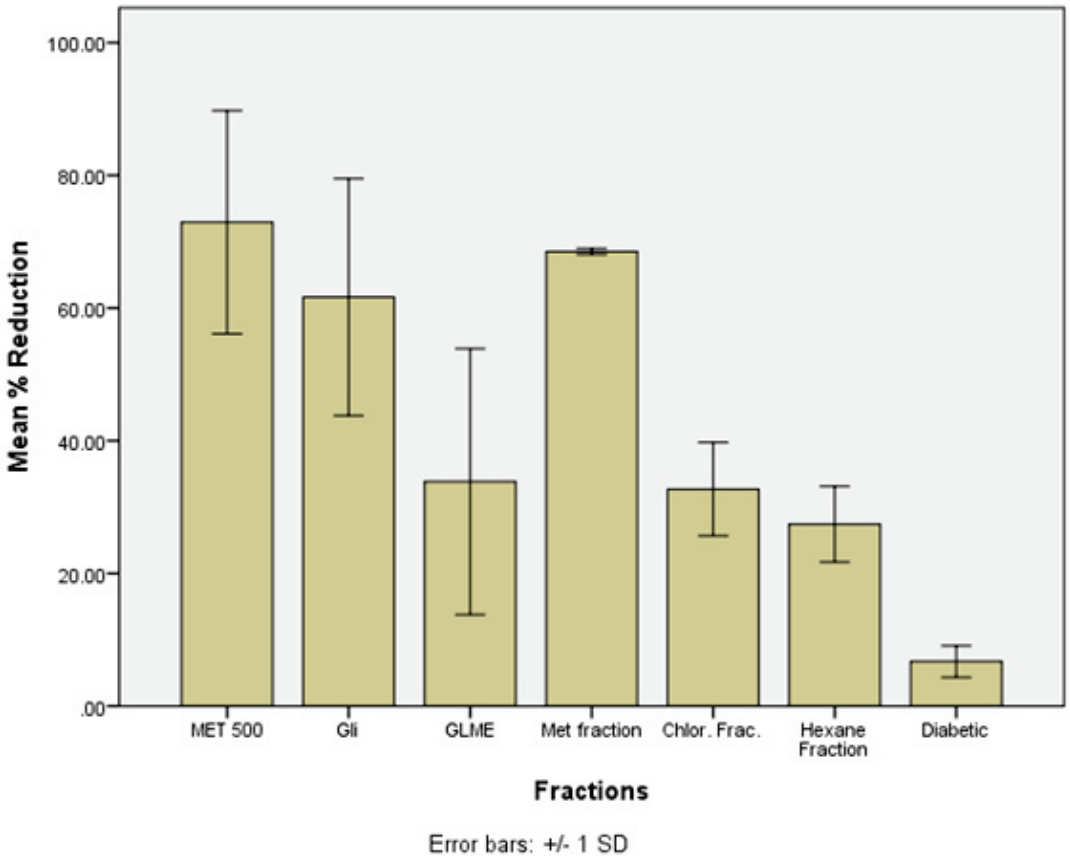

Figure 2: Results of anti-diabetic screening of methanolic extract of *G latifolium* fractions

Key
MET = Metformin treated diabetic rat,
GLI= Glibenclamide treated diabetic rat,
Diabet = Diabetic rat,
GLME = *Gongronema latifolium* methanol extract treated rat,
Met frac = Methanol fraction treated rat
Chlor frac= Chloroform fraction treated rat

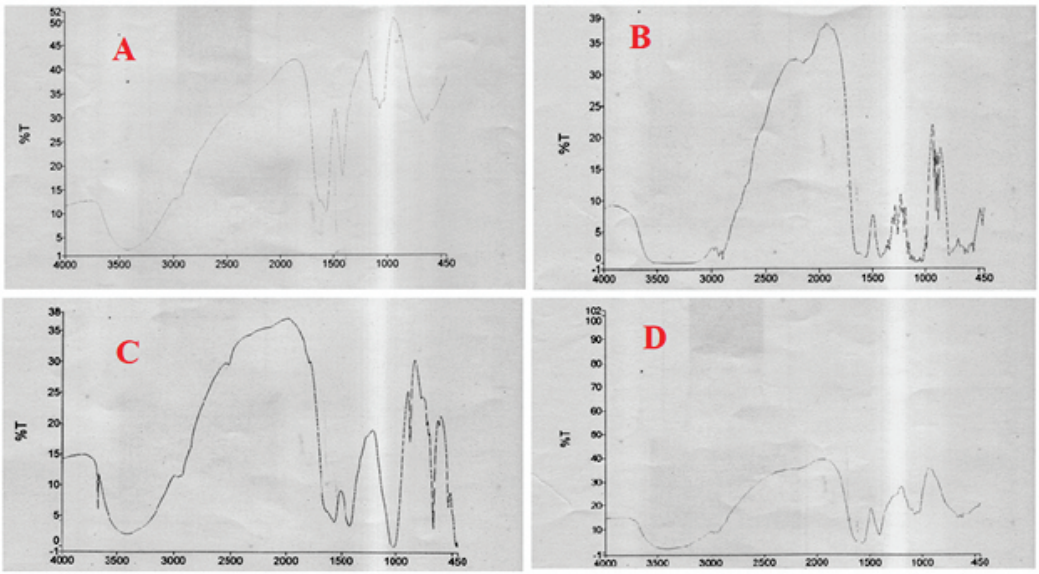

Figure 3 (A) IR Spectrum of GL (B) IR Spectrum of GL and Lactose (C) IR Spectrum of GL and Talc (D) IR Spectrum of GL and Gelatin

3.3 Solid dosage forms

The formulation of capsules resulted in transforming dark, bitter liquid herbal GL to capsules with high degree of elegance. The suppository of semi-purified *Gongronema latifolium* (GLPE) was prepared with glycerinated gelatin base formula. They were homogenous, dry, and smooth. Suppository was intended for very sick patients who cannot swallow drugs or who are vomiting.

GL capsules exhibited a good aesthetic appearance. The appearance was clean, dark red in colour. This will encourage patients to adhere to dosage regimens. The weight uniformity of capsules prepared using GL aqueous leaf extract was found to be within the pharmacopeial limits. None had percentage deviation of up to 10%. The disintegration times of GL capsules were 2 min. The time is within the BP specification of 30 min. The ultraviolet spectrophotometer scan obtained showed maximum wavelength of 274 for GL. Drug release profile of purified G. *latifolium* showed two sharp peaks of drug release line graph after 12 and 17 min while the aqueous was at 15 and 50 min (Figure 4 A and B).

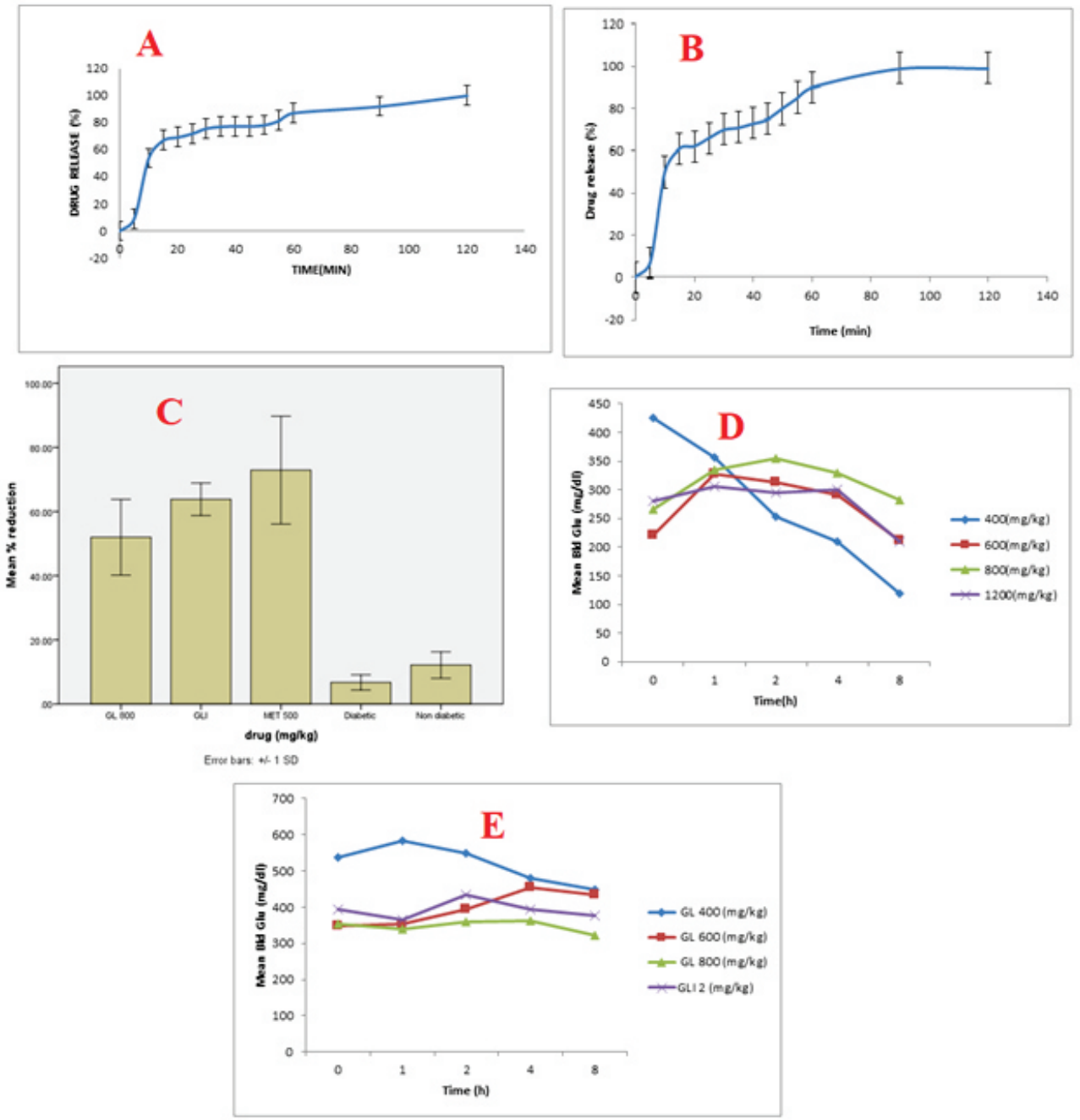

***Figure* 4** (A) Drug release profile of *Gongronema latifolium* semipurified (GLPE) **(B)** Drug

release profile of *Gongronema latifolium* leaf aqueous extract **(C)** Comparative anti-diabetic effect of G *latifolium* aqueous extract capsules with standards **(D)** Mean blood glucose time graph for various doses of GL capsules **(E)**The mean blood glucose level of GL suppository against time

3.3.1 Anti-diabetic effect of *Gongronema latifolium* aqueous leaf capsule

The comparative anti-diabetic effect of *G. latifolium* capsules with the standard drugs (Metformin and Glibenclamide) capsules is presented in Figure 4C. The standard capsules of Metformin and Glibenclamide had greater anti-diabetic effect than those of *G. latifolium*. The effect of doses of semi-purified GL capsules on STZ induced diabetic rats is displayed in Figure 4D. As shown in the graph, 400 mg/kg is the maximum dose that significantly reduced the mean blood glucose.

3.3.2 C-peptide content of treated-diabetic animals with GL aqueous leaf extract and semipurified capsules

Data obtained for quantitative determination of C-peptides in diabetic animals treated with GL aqueous leaf extract and semi-purified capsules is represented in Table 3. In GLPE capsule treatment group, maximum C- peptide value was obtained (115.76±1.54).

Table 3: The C-peptides content of animals treated with various drugs

Drugs/Extract	C-peptide (pM)
GLP E	115.76 ± 1.54
GLALE	86.25± 1.20
MET	33.98 ±0.08
GL	33.98 ± 0.58
Normal	2111.72± 1.61
Diabetic	0

3.3.3 Anti-diabetic effect of semi-purified *Gongronema latifolium* suppositories

Data obtained for mean blood glucose level showed that blood glucose level was not decreased by suppository for various concentrations of GL and standard Glibenclamide (Figure 4E).

4. Discussion

GL herbal plants have been shown to possess anti-diabetic activity. In this study, solid dosage forms of GL namely capsule and suppositories were formulated and evaluated. The result of phytochemical analysis of both aqueous and methanol extracts contains saponins, flavonoids, tannins and alkaloids. This result agrees with previous reports [2.11,12]. The result of preliminary screening clearly indicated that the administration of the GL produced anti-diabetic activity. The anti-diabetic screening of fractions of GL methanol extract (GLME) also showed that methanol fractions had the highest anti-diabetic activity (68 %) at the dose of 400 mg/kg body weight. Methanol fraction anti-diabetic result agrees with the report of Akah *et al.,*[11]. The observed anti-diabetic effect has been attributed to the regeneration of β-cells of the pancreas in alloxan-induced diabetic rats[11]. It could also be attributed to similar effects in STZ-induced diabetic animals, since both alloxan and STZ destroy pancreatic β-cells. Alloxan and its reduced product, dialuric acid indirectly induced diabetes by the formation of the superoxide radicals. These radicals ultimately cause rapid destruction of pancreatic β-cells[13]. On the other hand, STZ directly induced diabetes by entering pancreatic β-cells through the glucose transporter 2 (GLUT 2) channels in the plasma membrane, causing cellular toxicity. This leads to hypoinsulinemia and hyperglycemia in animals, inducing Type 1 diabetes [14].

Phytochemical results of the solvent fractions revealed that methanol fraction (MF) contained saponins, flavonoids, glycosides and carbohydrates. It was explained in literature that saponins and flavonoids are good anti-diabetic metabolites[11,12]. Chloroform and N-hexane fractions lacked these metabolites. It was suggested that the absence of these secondary metabolites could account for their non-significant anti-diabetic effect[11]. In another study, it was suggested that extracts of GL might reduce blood sugar level in a similar manner as insulin[2]. The extracts might bind to insulin receptors in the plasma membrane or cause the release of insulin from β-cells of pancreatic islets of Langerhans to initiate a signaling cascade that promote translocation and fusion of glucose transport system 4 (GLUT4) containing vesicles with plasma membrane to facilitate glucose transport into cells and organs[2].

Fourier transforms infrared spectroscopy results showed that GLME was compatible with lactose, talc and gelatin as shown in FTIR spectra. The compatibility studies suggested that the anti-hyperglycemic activity of the extracts will not be reduced by molecular interactions with chosen excipients. Therefore, lactose was chosen as a diluent for capsules and gelatin for suppositories.

Physical properties of powder mix of extract and excipients was poor for the powder blends had Hausner ratio > 1.25 and Carr's index of > 20 %. Powders with high interparticulate friction have Hausner ratios of >1.2 and Carr's index >20%, which indicate poor flow[15]. This showed GLALE and GLPE powders exhibited poor flow properties. Hence, their capsules cannot be produced in large scale with their optimized formula.

GL capsules exhibited a good aesthetic appearance. The

appearance was clean, dark red in colour. This will encourage patients to adhere to dosage regimens. Capsules have the advantage of being tasteless and easily administered[16]. Capsules passed all physico-chemical parameters including weight uniformity, disintegration time and dissolution tests. Hence further study on its anti-diabetic study was done. GL capsules had the lowest anti-diabetic activity on STZ induced diabetic rats even at a high dose of 800 mg/kg when compared with Glibenclamide and Metformin. This could be attributed to the low phytochemical constituents of the extract. Although it contains tannins, saponins and flavonoids as previously reported, the compositions of these constitiuents is low[2,11]. Glibenclamide reduces blood glucose by stimulating insulin secretion from pancreatic β- cells, while Metformin prevents insulin resistance without altering insulin receptor binding. It has also been reported that Metformin increases the intestinal glucose utilization.

C-peptide of GLPE capsule treatment group (115.76±1.54) was the highest. Oral administration of GL capsules significantly increased serum C-peptide in STZ- induced diabetic rats when compared with diabetic control rats. C-peptide level was significantly decreased in STZ- induced diabetic rats (0) due to destruction of β cells of pancrease. This is in line with the report by Akpaso that streptozotocin damages the pancreatic tissues, especially the Islet cells resulting in a prototype of Type 1 diabetes[17]. The measurement of C-peptide has been reported to be a valuable index of insulin secretion rather than insulin alone. The C-peptide levels increase was mainly an indication of continuing beta cell function and its relation to long-term control for diabetics [18]. This result agrees with report by Aka, that the anti-diabetic effect of GL has been attributed to the regeneration of β-cells of pancreas in alloxan-induced diabetic rats[11]. This can be explained that phytochemicals constituents of GL including saponins and flavonoids which cause regeneration of β-cells of pancreas thereby resulting in anti-diabetic activity.

The suppository of semi purified *Gongronema latifolium* (GLPE) was prepared. They were homogenous, dry, and smooth. Suppository was intended for very sick patients who cannot swallow drugs or who are vomiting. The poor reduction of mean blood glucose of GL suppository indicated that suppository is not an ideal dosage form for GL anti-diabetic herb as compared to GL capsules. Erratic and undesired absorption may be due to hydrophilic base used. This leads to ineffective lowering of blood glucose as obtained in capsules.

5. Conclusion

This research focused on formulation and evaluation of two solid dosage forms namely capsules, and suppositories of GL. The un-elegant, bitter liquid extract of GL can be transformed into solid dosage form. GL aqueous leaf extract and semi purified capsules were formulated with optimized formula. The capsules passed *pharmacopeial* requirements. The maximum anti-diabetic dose of semi-purified capsules of GL was 400 mg/kg. Suppositories of semi purified GL did not reduce hyperglycemia in STZ induced diabetes in animals. The result of the quantitative determination of C-peptides of GL treated animals confirmed that the treated groups had increased proliferation of β-cells. Therefore, one of the mechanisms of action of the GL anti-diabetic dosage forms was by proliferation and regeneration of β-cells that secrete insulin.

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