

Formulation and Evaluation of Lozenges Containing Freeze Dried Aqueous Extract of *Mangifera indica* for Management of Diabetes Mellitus

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Abstract

Objective: This study focused on the formulation of freeze dried *Mangifera indica*. (MI) lozenges dosage form, which enables proper dosing of MI for patients that are unable to swallow solid drugs.

Methods: Soft lozenges containing freeze dried aqueous extract of MI were prepared by melting Polyethylene glycol 1000(PEG 1000) to 70°C and gradually addition of MI with stirring after cooling. The mixture was poured into a lozenges mould. MI combinations with standard diabetic drugs namely Glibenclamide and Metformin lozenges were also prepared. Evaluation of lozenges including weight uniformity test, specific gravity test, dissolution rate test, *in-vitro* release test and physical stability tests were carried out. Investigation on anti-diabetic effect of lozenges containing MI individual and combinations with standard drugs on streptozotocin induced diabetic rats were carried out. The fasting blood sugar and change in weight of diabetic rats at the end of 14 days treatment were determined.

Results: All the lozenges passed weight uniformity test. Drug release profile showed that 100% MI was released after 1 min. The maximum anti-diabetic dose for MI lozenges was 250 mg/kg. The anti-diabetic effect of MI lozenges was 65.03±12.09 %. The anti-diabetic activity of individual MI lozenge was the same with the Glibenclamide (65.30±14.33). Weight gained by MI lozenges treatment group was significantly higher ($p<0.05$) than Glibenclamide standard group.

Conclusion: Lozenges containing MI freeze dried aqueous extract passed all parameters evaluated. The MI lozenges demonstrated significant anti-diabetic effect. MI combined with standard had no advantage over individual lozenges.

Keywords: Diabetes mellitus; *Mangifera indica*; Lozenges; Glibenclamide; Metformin.

Introduction

Mangifera indica. (family: *Anacardiaceae*) is a botanical name for the common mango. It is a large evergreen tree, perennial, 10-45 m high with a strong trunk of an average circumference of twelve to fourteen feet and heavy crown. It bears green linear oblong leaves. It is one of the important tropical fruit-trees in the world. The tree is grown widely in different parts of Africa and in Nigeria [1]. The chemical constituents of the plant include the flavonoids, polyphenolics, triterpenoids, isomangiferin, tannins and gallic acid derivatives. Mangiferin, a xanthone glycoside is

the major bioactive constituent. The bark is reported to contain protocatechic acid, catechin and mangiferin [2]. It also contains alanine, glycine, γ -aminobutyric acid, kinic acid, shikimic acid and the tetracyclic triterpenoidscycloart-24-en-3 β , 26diol, 3-ke-todammar-24 (E)-en-20S, 26-diol, C-24 epimers of cycloart-25 en 3 β ,24,27-triol and cycloartan-3 β ,24,27-triol [1].

There are many traditional and medicinal uses for different parts of MI. The leaves possess antibacterial, anti-ulcerogenic and anti-diabetic activities [3]. The pulp possesses numerous antibacterial properties against food borne bacteria [1].

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Diabetes Mellitus (DM) is a chronic disorder of carbohydrate, protein and lipid metabolism characterized by persistent elevations of fasting blood sugar over 200 mg/dl. DM is due to insufficient or complete cessation of insulin synthesis or secretion and peripheral resistance to insulin action [4]. There are also abnormalities in function and structure of blood vessels and nerves [5]. A number of studies have shown that Diabetes mellitus is also associated with oxidative stress, leading to an increased production of reactive oxygen species [6]. International Diabetes Federation estimated that about 425 million people are living with diabetes, with 5.1 million dying from it annually globally [7]. The prevalence was similar in both developed and developing countries. A recent meta-analysis reported that approximately 5.8% (about 6 million) of adult Nigerians are living with DM [8].

DM management concentrates on keeping blood sugar levels as close to normal ("euglycemia") as possible, without causing hypoglycaemia. This can usually be accomplished with diet, exercise, and use of appropriate medications (oral or insulin). These oral medications cause elevated cardiovascular risks, weight gain and/or hypoglycaemia [9] and are expensive. This is the reason for search for alternatives in herbal medicines which are safer, easier to obtain and have less adverse effects [10].

A few of the herbal medicinal treatments for DM have received scientific scrutiny, for which the World Health Organization (WHO) has also recommended attention [11]. Most plants extract are presented as dark bitter liquid with no consistent dosing. A solid dosage form will give proper dosing and longer expiration date. Many studies have been done on solid dosage form of herbal plants.

Lozenges are solid dosage forms that are intended to dissolve slowly in the mouth. They contain one or more medicaments in a base which may be flavoured [12,13]. They are usually intended for the treatment of local irritation or infections of the mouth or throat, but may contain active ingredients intended for systemic absorption after swallowing. They boost the time for drug to remain in the oral cavity thereby increasing bioavailability, lessen gastrointestinal discomfort and avoid first pass metabolism. The flavoured drug and ease of administration improve patient compliance [14]. There are two types of lozenges namely, soft and hard lozenges. Soft ones are popular because of ease of extemporaneous preparation and applicability to a wide variety and relatively low temperatures required for preparation [12].

Lozenges containing herbs have been formulated. Herbal lozenges can be made with a variety of different plant-based ingredients, depending on the intended use and desired effect [10]. A study was done in formulating hard lozenges of extract of eucalyptus and coleus aromaticus for the purpose of taste masking, crude drug release and consequent antimicrobial activity [15]. The formulated product showed inhibitory activity against non resistant *Candida albicans* infections thus providing a very good release matrix for the eucalyptus and coleus aromaticus combined extract [15]. Another study presented successful preparation of soft lozenges using *Pippier longum*, *Glycyrrhiza glabra* and Jaggery as a base for treatment of minor throat infections [16]. Garduchi lozenges is a hard candies which is made from sugar and widely used to treat fever, respiratory issues, diabetes, anaemia, and cardiac diseases [17]. However, no studies have been done on lozenges containing MI leaf extract for anti-diabetic patient. This work focused on formulation of lozenges containing freeze dried aqueous leaf extract of MI that

has folkloric and scientific use as anti-diabetic.

Materials and methods

Materials

Chemicals and drugs

Starch soluble (BDH, England), Normal saline (Juhel, Nigeria), Metformin Hydrochloride (Sigma Aldrich, Germany), Glibenclamide (Santa Cruz, USA), distilled water, Streptozotocin SO130 (Sigma Aldrich Co, St Liouis USA), polyethelene glycol 1000 (Merk, Germany), formaldehyde (M&B England), Chloroform (Sigma Aldrich, Germany), Olive oil, Fehling's reagent I and II, Lead acetate (Merck, Germany), Sulphuric acid (Sigma Aldrich USA), Sodium hydroxide (Merck), Iodine solution, Wagner's reagent, Dragendoff's reagent.

Animals

One hundred and fifty (150) inbred albino wistar male rats (weighing 100-140 g) were bred in the Laboratory Animal unit of Faculty of Pharmaceutical Science and used for some of the experiments. The environmental temperature varied between 25 and 30°C and the lighting period was between 15 and 17 h daily. The rats were given clean drinking water and fed with standard commercial pelletized growers feed (Vital Feed, Nigeria).

All procedures were performed in compliance with relevant laws and guidelines approved by Animal Research Ethics Committee of the University.

Aqueous extraction of *Mangifera indica*. (MI) leaves

Mangifera indica. leaves was sourced once from MI tree in Enugu East, Enugu state. The washed MI leaves was shade dried in a room at 29°C. A 200 g powder of shade dried MI leaves was extracted by immersing it in distilled water for 48 h at 28°C. The soaked leaves was shaken at regular intervals of 2 h and extract was filtered with muslim cloth and then with filter paper (No 1 whatmann). The extract was freeze-dried and stored in the freezer.

Phytochemical tests

The MI extract was analyzed for the presence of phytochemical constituents using the methods described in literature [18].

Preliminary screening of anti-diabetic activity of MI extract

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 (2-5 groups) 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 control group (1) and diabetic group (5) received 2 ml/kg of normal saline. Animals in group 2 received MI (250 mg/kg) while groups 3 and 4 received standard drugs 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 as stated in Equation 1.

$$\text{Percentage Blood Glucose Reduction} = \frac{1 - F_f}{F_i} \times 100 \quad (1)$$

Where

F_f = Final mean blood glucose; F_i = Initial mean blood glucose.

Formulation of soft lozenges

The lozenges mold was prepared and calibrated by melting sufficient Polyethylene Glycol 1000 (PEG) to fill six molds. The average weight of the six lozenges was determined.

The weight of PEG was calculated as shown in Table 1 and taking the density factor of extract as 0.7 [12]. The accurately weighed PEG was placed in 500 mL beaker and was gently heated directly on a heater till temperature reaches 70°C over 5 min period. The freeze dried aqueous extract was triturated and then added to the melted PEG. Homogenous mixture was obtained by a thorough mixing process using constant temperature magnetic stirrer. The mixture melt was then poured into the lozenges mold and allowed to cool. The lozenges were stored in a refrigerator.

Evaluation of anti-diabetic leaf extract of lozenges

The evaluation of lozenges containing MI includes physical observations, weight uniformity test, specific gravity, dissolution test, *In-vitro* drug release and physical stability.

Physical observation such as colour, clarity, surface texture and appearance of the lozenges containing MI was done visually.

Weight uniformity test

Weight uniformity test was performed for 20 MI lozenges and MI lozenges combined with standard. The percent weight deviation of lozenges was calculated as described in literature [19].

Table 1: Formula for MI lozenges.

Drug/excipients	Qty/1lozenge	Qty/4 in 1 lozenge
M. Indica (Rat Wt 130 mg)	250 mg/kg	4X250 mg/kg
PEG1000 Mold size (1.3g)	q.s	qs

Specific gravity determination

Specific gravity was determined for lozenges containing MI using a 10 ml beaker containing 5 ml volume of water. A weighed MI lozenges was placed in the beaker and final volume noted. The change in the volume was taken as volume occupied by lozenges. Three replicates were carried out. The specific gravity was calculated as stated in Equation 2.

$$\text{Specific Gravity} = \frac{\text{weight of the lozenges}}{\text{volume of the lozenges}} \quad (2)$$

Dissolution test of extract lozenges

A 200 ml quantity of distilled water was heated to 37°C on a magnetic stirrer unit (Searchtech, China) set at about 50 rpm. A lozenge was added to the water and observation of extent of melting was recorded with time. Three replicates were done.

Determination of maximum wave length and *In-vitro* release studies

A 100 mg quantity of MI 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. Thereafter, different concentrations were used to prepare a Beer's plot of MI using the appropriately determined λ max.

A 900 ml volume of distilled water was poured into a beaker placed on a heater magnetic stirrer. The water was thermostatically maintained at 37°C at a rotational speed of 50 rpm. The lozenges was introduced into the beaker. Thereafter, samples of 5 ml were withdrawn at 2 min predetermined intervals using a pipette. Replacement of 5 ml of distilled water was done after each withdrawal. The withdrawn samples were analyzed in a UV-spectrophotometer at predetermined wavelength of 258 nm (λ max). Cumulative percentage drug release was calculated using an equation obtained from a standard calibration curve.

Physical stability

Ten lozenges for each extract were set apart in refrigerator of 1.7-3.3°C temperature for physical stability observation. Observation of the lozenges for signs of discoloration, dryness, cracking, mottling and mold growth were done on weekly basis for eight weeks.

Dose-related anti-diabetic response of MI lozenges

Thirty-six rats of male sex weighing 120-150 g were randomly divided into 6 groups (1-6) of six rats per group and fasted for 14 h. Each rat in each group was intra-peritoneally injected with accurately determined volume of STZ (65 mg/kg) in distilled water. The animals were fed with standard pellet diet 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 lozenges containing freeze dried MI was orally administered at doses of 250, 375 mg/kg to groups 2 and 3 respectively. Then standard Glibenclamide (2 mg/kg) and Metformin (500 mg/kg) was administered to groups 4 and 5. The control group (1) and diabetic group (6) received 2 ml/kg of normal saline orally. Blood glucose of the treated rats was measured at 0, 1, 2, 4 and 8 h using Accu-check Advantage glucometer (Roche). The percentage glucose reduction was calculated according to Equation 1.

Effect of combination extracts of MI and standard lozenges on STZ-induced diabetic rats

Forty two healthy male albino rats (100-150 g) were randomly selected and housed in seven groups (1-7). The animals were fed with standard pellet diet and water *ad libitum*. The animals were fasted 14 h before induction of diabetes (2-7 groups) by Intraperitoneal (IP) injecting a single dose of Streptozotocin (STZ) (65 mg/kg body weight). The accurately weighed STZ was dissolved in distilled water and injected immediately within few minutes 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 7 were designated normal and diabetic controls and received 2.0 ml/kg body weight of normal saline. Test groups 2-4 received MI (250 mg/kg), MI and GLI (250: 2 mg/kg), MI and MET (250: 500 mg/kg) lozenges respectively. In groups 5 and 6, standard drug Glibenclamide (GLI) (2 mg/kg) and Metformin (500 mg/kg) lozenges were given respectively. The blood glucose of all the rats was measured at predetermined times of 0,1,2,4,8 h. and the percentage reductions were determined.

Sub-acute treatment of STZ- induced diabetic rat with aqueous MI leaf extract lozenges

Thirty rats of male sex weighing 120-150 g were randomly divided into 5 groups (1-5) and fasted for 14 h. Each rat in each group was intra-peritoneally injected with accurately determined volume of Streptozotocin STZ (65 mg/kg) in distilled water. The animals were fed with standard pellet diet 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 lozenges containing MI accurately prepared were orally administered at doses of 250 mg/kg to group 2. Standard drugs Glibenclamide (2 mg/kg) and Metformin (500 mg/kg) were administered to groups 3 and 4. The control group 1 and diabetic group 5 received 2 ml/kg of normal saline orally. The treatment of STZ induced diabetic rat was done for 14 days. At the end of treatment, the weight of rats and the blood glucose for each group was measured.

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. p values less than 0.05 was considered significant.

Results

Herbal plants have been shown to possess anti-diabetic activity. In this study, Lozenges solid dosage forms containing freeze dried aqueous extract of MI were formulated and evaluated.

Phytochemical results

The phytochemical results of aqueous leaves extract of MI are shown in (Table 2). The table showed that MI extracts contain majorly glycoside.

Table 2: Phytochemical results of aqueous leaves extract of MI.

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

Key: + = small ++ = moderate +++ = high (quantified subjectively).

Preliminary anti-diabetic screening results

The percentage blood glucose reduction of MI aqueous leaf extract before formulation into lozenges was 67.85% as shown in (Figure 1). This value is greater than Glibenclamide standard anti-diabetic drug but was not significant.

Lozenges solid dosage forms

The formulation of lozenges resulted in transforming dark, bitter liquid herbal MI extract to lozenges solid dosage form with good appearance. The appearance of lozenges was dark brown, smooth oily surface with clarity.

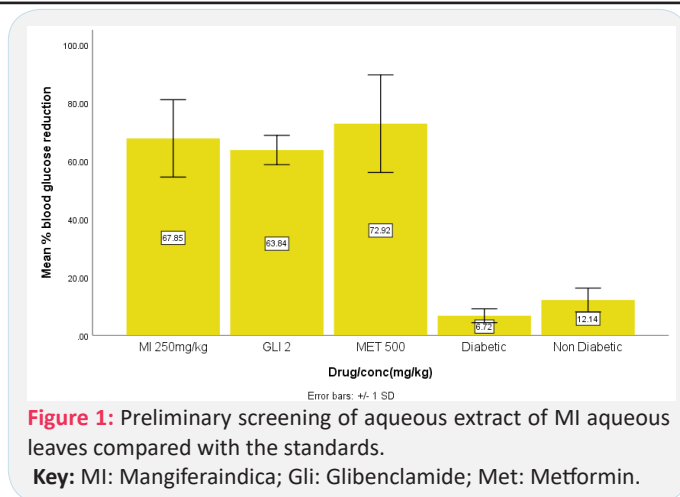


Figure 1: Preliminary screening of aqueous extract of MI aqueous leaves compared with the standards.

Key: MI: Mangiferaindica; Gli: Glibenclamide; Met: Metformin.

The weight uniformity of MI lozenges was found to be within the pharmacopeial limits. None had percentage deviation of up to ±10%. The specific gravity of MI lozenges was 0.465 and was not significantly different from that of Metformin and Glibenclamide which were 0.485 and 0.478 respectively. The dissolution time of MI lozenges was 10 min. The *in-vitro* drug release of MI lozenges showed 100% release of MI after 10 min as shown in (Figure 3).

Ultra violet spectrophotometer scan of MI

Figure 2 shows the scan of MI aqueous extract using ultra-violet spectrophotometer. The scan is a graph of absorbance against wavelength. A sharp mark of maximum wavelength is at 285 nm. The maximum wavelength 285 nm was used to measure concentration of the extract during *in-vitro* drug release.

Drug release profiles of aqueous leaf extract of MI lozenges

The drug release profiles of the aqueous leaf extract of lozenges showed that most of the MI extract was released after 8 min (Figure 3).

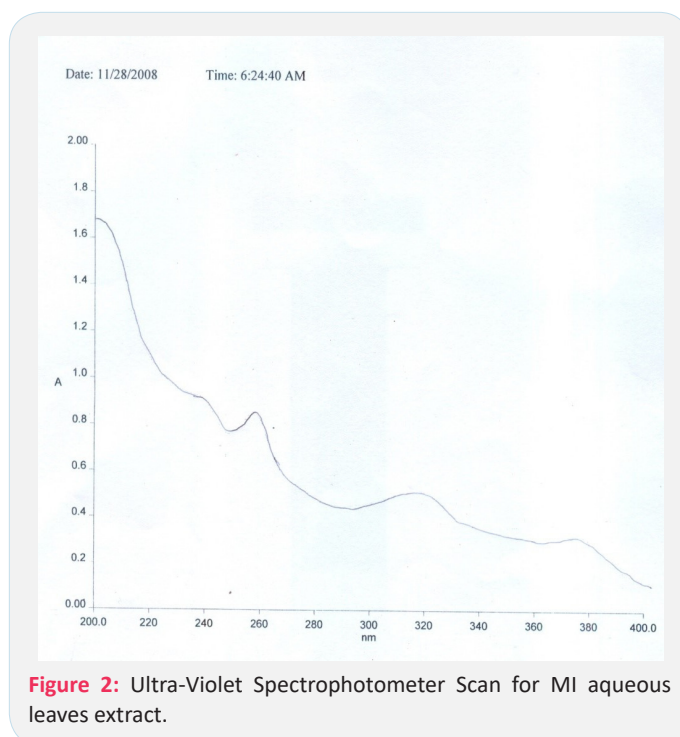


Figure 2: Ultra-Violet Spectrophotometer Scan for MI aqueous leaves extract.

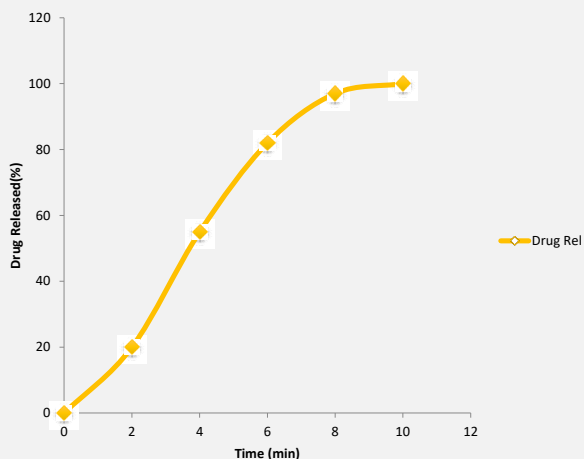


Figure 3: Drug release profiles of Lozenges containing MI aqueous leaf extracts.

Physical stability

After eight weeks of observation, the MI lozenges showed no signs of discoloration, dryness, cracking and mottling mold growth.

Various concentration of MI blood glucose- time related response

The blood glucose-time response of different concentrations of MI lozenges is presented in (Figure 4). The graph shows that 250 mg/kg has maximal blood glucose reduction.

MI lozenges effect on blood glucose of STZ- induced diabetic rats

The MI lozenges effect on STZ induced diabetes in wistar rats is shown in Figure 5. The percentage blood glucose reduction of MI is the same with Glibenclamide but lower than Metformin. There was no significant difference between MI aqueous leaf extract and standard drugs (Met and GLI) lozenges $P > 0.05$. The anti-diabetic effect of formulated 250 mg/kg MI lozenges was 65.03% which is lower than the anti-diabetic of MI extract alone 67.85%.

Effect of combination of MI leaf aqueous extract and standard lozenges on diabetic rats

Figure 6 displays the effect of combination of MI and standard drugs lozenges on the percentage reductions of blood glucose of STZ induced diabetic rats. The combination of MI and Metformin lozenges had almost the same percentage reduction with MI. There was a significant difference between MI individual and MI combination with Glibenclamide (MI and GLI) lozenges on blood glucose reduction ($p < 0.05$). The blood glucose reduction effect of combination of MI and standard was not positively significant.

Effect of MI lozenges on feeding habit of diabetic rats

Treatment of diabetic rats with individual lozenges was carried out for two weeks. The feeding habit during the course of treatment is shown in (Table 3). Among the treatment groups, there was no significant difference between MI and standard drugs food fraction per day. On the other hand, diabetic group had the lowest food intake and more water intake.

At the end of treatment, changes in weights were measured. The change in weights is shown in (Table 4). Diabetic rat had

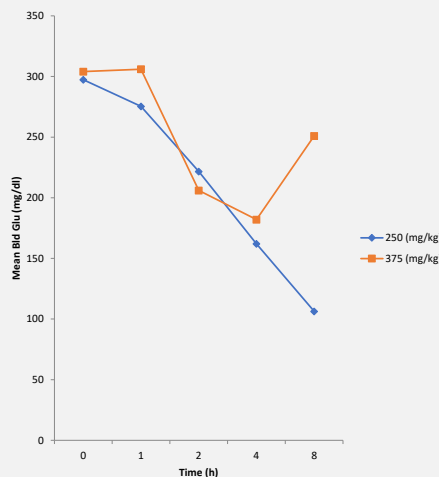


Figure 4: Blood glucose against time for various concentrations of MI Lozenges.

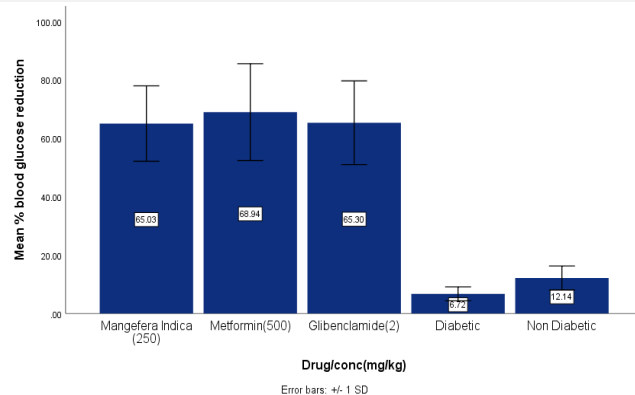


Figure 5: Comparative study of anti-diabetic effect of MI lozenges with standard lozenges.

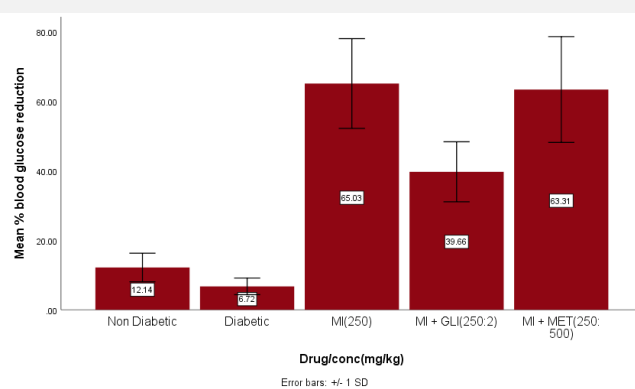


Figure 6: The percentage blood glucose reductions of various MI combined with standard lozenges.

weight loss. However, MI lozenges treated animals had significant increase in weight when compared to the standard treatment groups.

Effect of MI lozenges on blood glucose after a 14 days treatment

The effect of MI lozenges treatment on STZ induced diabetic rats after 14 days of treatment is presented in (Figure 7). There was a great improvement in blood glucose reduction after 14 days treatment in MI treatment group as shown in the graph, MI treatment group blood glucose reduction was not significantly different from standard (Metformin and Glibenclamide)

$p > 0.05$. Moreover, the final fasting blood glucose levels of all treatment groups after 14 days of therapy were in comparison with normal control.

Table 3: Feeding habit of the treated rats using various lozenges.

DRUG	Day 1		Day 2		Day 3		Day 4		Day 5		Day 6		Day 7		Day 8		Day 9		Day 10		Day 11		Day 12		Day 13		Day 14	
	F(g)	W(ml)	F(g)	W(ml)	F(g)	W(ml)	F(g)	W(ml)	F(g)	W(ml)	F(g)	W(ml)	F(g)	W(ml)	F(g)	W(ml)	F(g)	W(ml)	F(g)	W(ml)	F(g)	W(ml)	F(g)	W(ml)	F(g)	W(ml)	F(g)	W(ml)
ND	16.82	50	17	50	29.8	52	25	60	25	60	25	30	25	30	20	20	15.8	100	20	50	12	50	13.4	80	28	80	30	80
DIA	44.3	75	22	50	39.5	50	40	100	20	50	20	50	20	50	20.5	55	21.2	60	27	100	28.4	100	25	100	33	95	32.3	100
MI	26.7	51.6	26.7	66.7	15.3	33.3	26.7	66.7	20	50	20	100	13.5	100	9.2	100	22	50	34.7	100	34.7	100	34.9	100	35	100	49	100
GLI	24.7	70	16	50	24.5	50	30	100	30	100	30	100	30	100	46	100	33	130	40.8	100	40.8	100	41	100	55	110	48	100
MET	10	140	26	100	22	100	44.3	80	42	90	43	95	45	98	47	100	50	100	52	100	55	100	57	100	62	100	58.6	100

Table 4: Changes in weight of rats after 14 days treatment.

DRUGS /Conc.(mg/kg)	Initial day Weight(g) Average	Final Day Weight(g) Average	Change in Weight (g) Average
MI (250)	106.27	116.67	10.4
Diabetic	121.67	102.47	-20.33
Met(500)	113.67	122.93	9.27
GLi (2)	111.67	116.33	4.67
Non Diabetic	115	123.57	8.57

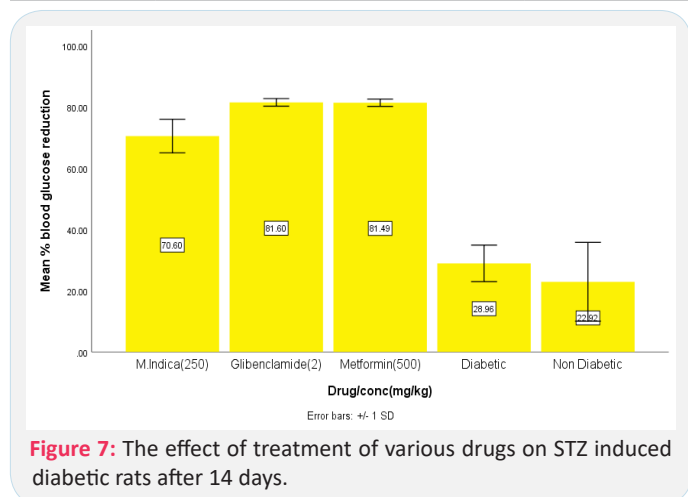


Figure 7: The effect of treatment of various drugs on STZ induced diabetic rats after 14 days.

Discussion

Many herbal plants have been used for treatment of various diseases. Many have undergone scientist scrutiny. *Mangifera indica* plant which has folkloric use has been scrutinized for many parameters such as anti-diabetic, antibacterial and anti-ulcerogenic [1]. The percentage blood glucose reduction of MI freeze dried aqueous leaf extract was 67.85% which shows that MI aqueous extract has anti-diabetic effect. It is normally presented as a dark liquid substances. Formulation of lozenges solid form containing freeze dried aqueous extract of MI will enable proper consistent dosing.

Lozenges are solid preparations that are intended to dissolve slowly in the mouth. The lozenges solid form will enable proper consistent dosing of MI. The MI lozenges formulated was dark, brown, smooth oily surface with clarity. The lozenge will stay for a period in the mouth which will boost the time for drug to remain in the oral cavity thereby increasing bioavailability, lessen gastrointestinal discomfort and avoid first pass metabolism [14]. The good appearance of MI lozenge and ease of administration will encourage patients to adhere to therapy.

The weight uniformity of MI lozenges showed that none had percentage deviation of up to $\pm 10\%$. This depict that lozenges had content uniformity. The *in-vitro* drug release of the lozenges showed 100% release of the drug after 10 min. The *in-vitro* drug release time (10 min) of MI correspond to acceptable time release of soft lozenges [10]. It is also in agreement *in-vitro* time release of Eucalyptus oil and coleus aromatic oil lozenges [15]. This suggests that fast MI release from the lozenges will occur *in-vivo*. *In-vitro* drug release time is an important parameter to evaluate in herbal lozenges as it can affect the release of active ingredients and the efficacy of the product. Various physical characteristics, type and number of used excipients and environmental conditions during storage influence *in-vitro* drug release time of lozenges [10]. This fast release of MI will infer that fast therapeutic outcome of reduction of blood sugar in-patient with diabetes. Consequently, patient's adherence to therapy will be 100% for they received good clinical outcome in a short time. Apart from this, this formulation will be an intervention to improve health related quality of life of patients in terms of general health, social functioning, mental health scales, energy and daily activities. In addition, economic outcomes will result for significant cost savings in buying one lozenge to handle diabetes than buying two drugs in management of patients with chronic diseases when blood sugar refused to be controlled.

The physical stability studies showed no changes in MI lozenges after storage for eight weeks in refrigerator. This could be attributed to proper selection of polyethylene glycol (PEG 1000) base and optimized formula. It may also infer that no incompatibility existed between MI aqueous extract and base PEG 1000. This is in agreement with study done by Kishori where no physical incompatibility existed in lozenges.

The percentage blood glucose reduction of MI lozenges (250 mg/kg) was 65.03%. There was no significant difference between MI aqueous leaf extract and standard drugs (Met and GLI) lozenges ($P < 0.05$). The marked reduction in blood glucose could be attributed to effect of phytochemical composition. MI may regenerate β -cells and other extra pancreatic mechanism such as insulin mimetic action and oxidative stress attenuation [2].

The combination of MI and Metformin lozenges was similar to individual MI lozenges. On the other hand the combination of MI and glibenclamide had lower blood glucose reduction of 40% which was significantly different. The blood glucose reduction effect of combination of MI and standard has no positive result. Therefore, preparation of individual MI lozenges will benefit the users and producer.

After treatment of diabetic rat for 14 days, there was no significant difference between MI and standard drugs food fraction per day. This may be due to regenerated β - cells that caused increased appetite [21]. MI lozenges treated animals had significant increase in weight compared to the standard treatment groups. This weight gain indicated that the treatment allowed the tissues to access the glucose both to supply energy and to build tissue materials required for growth. Diabetic rat had weight loss which is attributed to loss in muscles and adipose tissues due to increased metabolic rate and glycosuria [22,23].

Moreover, the final fasting blood glucose levels of all treatment groups after 14 days of therapy were in comparison with normal control. This can be due to regeneration of damaged β cells by streptozotocin [21]. This in turn caused increased insulin secretion capacity and other extra-pancreatic mechanism. The extracts of MI lozenges also possess phytochemicals that may reduce the intestinal glucose absorption in the gut by various mechanisms [3].

Conclusion

The formulation of lozenges is a straightforward and efficient process. Medicated lozenges are ideal dosage forms for patients who are unable to swallow solid dosage drugs due to their ease of administration, patient compliance, convenience and comfort during treatment.

Lozenges dosage form of freeze dried aqueous leaves extract of MI (250 mg/kg) was successfully prepared. The lozenges physicochemical parameters were within acceptable range according to official pharmacopeia. The anti-diabetic activity of MI lozenges was significantly high. MI combined with standards had no advantage over individual lozenges. MI Lozenges will enhance adherence to therapy for patients that have difficulty in swallowing MI capsules or tablet hence improve adherence and therapeutic outcome.

References

- Shah KA, Patel MB, Parmar PK. *Mangifera indica* (Mango). Pharmacognosy Rev. 2010; 4(7): 42- 48.
- Muruganandan S, Srinivasan K, Gupta Lal J. Effect of Mangleferin on Hyperglycemia and Atherogenicity in Streptozotocin Diabetic rats. Journal of Ethnopharmacology. 2005; 97: 497-501.
- Bhowmik A, Khan AL, Akhter M and Rokey B. Studies on the Antidiabetic Effects of *Mangifera indica* Stem-Barks and Leaves on nondiabetic Type 1 and Type 2 Diabetic Model Rats. Bangladesh J pharmacol. 2009; 4: 110-114. <http://www.bdjpharmacol.com>.
- Murray M, Pizzorno J. Encyclopedia of Natural Medicine. 2nd Edition, Prima Health Publishing, Ruckling, USA. 1997; Pp 401.
- Rao MU, Sreenivasulu M, Chengaiah-reddy KJ, Chetty CM. Herbal medicines for Diabetes mellitus. International journal of PharmTech Research. 2010; 2(3): 1883-1892.
- Ugochukwu NH, Babady NE, Cobourne M, Gasset SR. The effect of Gongronemalatifolium Extracts on Serum Lipid Profile and Oxidative Stress in Hepatocytes of Diabetic Rats. J. Biosci. 2003; 8(1): 1-5. [http:// link.springer.com//](http://link.springer.com//)
- International Diabetes Federation. Diabetes in Africa. 2019.
- Ugwu E, Young E, Nkpozi M. Diabetes Care, Knowledge and Practice Among Primary Care Physicians in Southeast Nigeria: A Cross-Sectional Study. BMC Fam Pract. 2020; 21: 128. [//doi.org/10.1186/s12875-020-01202-0](https://doi.org/10.1186/s12875-020-01202-0)
- Maroo J, Vasu VT, Aalinkeel R, Gupta S. Glucose Lowering Effect of Aqueous Extract of *EincostemmalittoraleBlume* in Diabetes; A Possible Mechanism of Action. Journal of Ethno pharmacology. 2008; 1(3): 317-320.
- Parul V, Harshita J, Shipra S, Nitin N. Development And Evaluation Of Herbal Lozenges, Career Point International Journal of Research(CPIJR). 2022; 1(2): 53-68.
- World Health Organization. WHO Regional office for the Western Pacific Research Guidelines or evaluating the safety and efficacy of herbal medicines.1993.
- United State Pharmacist. Pharmacopeia. 2009; 1108-1109.
- Smith JD. Diabetes Mellitus In Comprehensive Pharmacy Review for NCLE, Shargel L, Mutnick AH, Sournay PF, Swanson LN (eds), 8th edition, Lippincott Williams and Wilkins Philadelphia. 2013; 930-953.
- Deshmukh A, Kolpyakwar G, Turre T, Kale V, Umekar M, et al. Utilization of Polyherbal Drug to Formulate Lozenges for Treatment of Various Disease. International Journal Of Innovative Research In Technology. 2022; 9(7): 2349-6002.
- Anand B, Thomas I, Beena P, Abraham E, Formulation and Evaluation of Herbal Lozenges Containing Eucalyptus Oil and Coleus Aromaticus Oil. American Journal of PharmTech Research. 2018; 8(1): 316 -322. [https://: www.ajptr.com](https://www.ajptr.com).
- Mishra KK, Tasneem K, Jain V, Mhajan SC. Formulation and evaluation of herbal lozenges, Journal of Drug Delivery and Therapeutics. 2017; 7(7): 87-89. <http://jddtonline.inf>
- Kadan PA, Bavage SB, Lonikar NB, Kokare PS. Development and Evaluation of Herbal (Guduchi) Hard Candy Lozenges. International Journal of Research Publication and Reviews. 2023; 4(6): 730-739. [http// www.ijrpr.com](http://www.ijrpr.com).
- Evans WC. Trease and Evans Pharmacognosy (15th. ed). WB Saunders Company, UK. 2000; 41(191): 491-20.
- Talman FA. Tablets and Capsules. In: Bentley's Textbook of Pharmaceutics, Rawlins EA (Ed) 8th ed. East Sussex, BaillierTindall. 1996; 313-314.
- Kishori S, More V, Kimaya, Maniyar M. Formulation and Evaluation of Lozenges Containing Extracts of Capparis Decidua To Treat Toothache. Eur. Chem. Bull. 2023; 12(10): 2113-2120. DOI: 10.48047/ecb/2023.12.si10.00253
- Akpaso MI, Atangwho IJ, Akpantah A, Fischer VA, Igiri AO, et al. Effect of Combined Leaf Extracts of *Vernoniaamygdalina* (Bitter Leaf) and *Gongronemalatifolium* (Utazi) on the pancreatic- β Cells of streptozotocin- induced Diabetic Rats. British Journal of Medicine and Medical Research1. 2011; (1): 24-34. [http// www.sciencedomain.org](http://www.sciencedomain.org)
- Iweala EE, Uhegbu FO, Adesanoye OA. Biochemical Effects of Leaf Extracts of *Gongronemalatifolium* and Selenium Supplementation in Alloxan Induced Diabetic Rats. J Pharmacognosy phototherapy. 2013; 5(5): 91-97.
- Akah PA, Alemij JA, Okoye TC, Offiah NV. Effects of *Vernoniaamygdalina* on Biochemical and Hematological Parameters in Diabetic Rats. Asian Journal of Medical Sciences. 2009; 1(3): 108-113.