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Formulations and Evaluations of Lozenges Containing Freeze Dried Aqueous Extract of Vernonia Amygdalina Combined with Glibenclamide or Metformin for Management of Diabetes Mellitus

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Abstract

Objective: The aim is to formulate freeze dried Vernonia Amygdalina (VA) lozenges dosage form. VA lozenges combined with Glibenclamide and Metformin standard anti-diabetic effect will also be studied. This will enable proper dosing of VA for patients that are unable to swallow solid drugs.

Methods: Lozenges containing freeze dried aqueous leaf extract of Veronica Amygdaline (VA) were prepared with by conventional techniques. Soft Lozenges were prepared by melting polyethylene glycol 1000 (PEG 1000) to 70°C and gradual addition of VA with stirring after cooling to 40°C. The mixture was poured into lozenges mould. VA combinations with the standard diabetic drugs namely Glibenclamide and Metformin lozenges were also prepared. The physicochemical evaluations of lozenges namely physical examination, weight uniformity, specific gravity, dissolution, in-vitro release test and physical stability tests were carried out. Investigation on anti-diabetic effect of lozenges containing VA individual and combinations with standard drugs on streptozotocin-induced diabetic rats were carried out. Effect on fasting blood sugar and change in weight of treated diabetic rats at end of 14 days treatment were determined.

Results: The physical examination of lozenges containing VA individual and combined was satisfactory. Thickness of lozenges was ranged from 3-4 cm. All the lozenges passed weight uniformity tests and dissolved after 5 min. Drug release profile showed that 100% of VA was released after 2 min. The maximum anti-diabetic dose for VA lozenges was 150 mg/kg. The antidiabetic effect of VA lozenges was 69.95±13.34%. Combination of VA and both standard drugs had no significant effect on anti-diabetic activity when compared with VA individual ($p>0.05$). There was remarkable fasting blood sugar reduction in VA lozenges treatment group. Weight gained by VA lozenges treatment group was 7.8±2.5 and was significantly higher ($p>0.05$) than Glibenclamide standard group at end of 14 days treatment.

Conclusion: Based on the findings from this investigation, lozenges containing VA freeze dried aqueous extract both individual and combined passed all pharmacopeia requirements evaluated. The VA lozenges demonstrated significant anti-diabetic activity. VA combined with standards had no advantage over individual VA lozenges.

Keywords: Lozenges; Vernonia amygdalina; Glibenclamide; Metformin; Diabeic mellitus.

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Introduction

Veronica Amygdalina (family *Asteraceae*) is a shrub that grows up to 23 feet tall. It is commonly called bitter leaf in English and has other names namely *onugbu* (Igbo), *ewuro* (Yoruba) and *etidot* (Ibibio) [1]. *V amygdalina* has been reported for pharmacological effect such as anti-oxidant [2]. Anhancement of immune system, decreased blood glucose [3]. And anthelminthic and anti-parasitic properties [4]. The reported bioactive principles include saponins, flavonoids, glycosides, oxalates, phytates and tannins [5]. These compounds have been shown to be responsible for the observed biological activities as seen in flavonoids that possess antioxidant activity [6].

Despite the efficacy of extracts of various anti diabetic herbs presented in the literature, capsules formulations that give specific dose are the common in the pharmaceutical market [7]. However, the capsules and tablets are not suitable for patients who have difficulty in swallowing tablets and capsules. Lozenges can be used by patients that are unable to swallow capsules/tablets. Lozenges are described as solid dosage forms preparations that are intended to dissolve slowly in the mouth. They contain one or more medicaments and flavoured sweetened base. 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. Compressed lozenges are often referred to as troches [8-11]. There are two types of lozenges namely, soft and hard lozenges. Soft ones are popular because of ease of extemperoneous preparation and applicability to a wide variety of drugs [8]. The lozenges can be prepared as a personalized medicine for very sick patients and children that are unable to swallow capsules/tablets. This study focused on formulation of lozenges containing freeze dried aqueous leaf extracts of *V amygdalina* that is commercially available as capsules dosage form. Preparation and evaluation of lozenges containing combination of freeze dried aqueous extracts of VA and standards: Glibenclamide and Metformin lozenges was also studied.

Materials

Chemicals and drugs

Lactose (Santa Cruz, USA), Normal saline (Juhel, Nigeria), Metformin Hydrochloride (Sigma Aldrich, Germany), Glibenclamide (Santa Cruz, USA), Streptozotocin SO130 (Sigma Aldrich Co, St Lious, USA), olive oil, Fehling's reagent i and ii, lead acetate, sulphuric acid (Sigma Aldrich USa), sodium hydroxide (Meck), Iodine solution, wgner's reagent , Dragendoff's reagent.

Instruments

Analytical weighing balance (Sartorius AG, Germany), Top loading balance (S.Mettler- China), sonicator, Accu-check machine advantage II and strips (Roche, Germany). Magnetic stirrer unit (Searchtech, China), Ultra-Violet (UV) spectrophotometer (PerkinElmer Lambda 35, Germany), vernia calipar.

Animals

Two hundred (200) 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 and were 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.

Methods

Aqueous extraction of *Vernonia Amygdaline* (VA)

Fresh leaves were sourced from market in Enugu East, Enugu state. The washed VA leaves were air dried at room temperature of 28°C. They were pulverized into fine powder using a blender, sealed in polythene bags and stored at 4°C in refrigerator. Two hundred grams (200 g) of powdered dry leaves of VA was extracted by immersing it in two litres (2L) of distilled water for 48 h at 27°C. The extract was filtered with muslim cloth to remove the particulate matter before filtration through a filter paper (No 1 what Mann). The resulting extract was freeze- dried and stored in the freezer.

Phyto chemical tests

The VA extract was analyzed for the presence of phyto chemical constituents using the methods described in literature [12].

Preliminary screening for anti-diabetic activity of aqueous extract

Thirty male albino wistar rats (100-130 g) were randomly selected and housed in five groups [1-5]. They were acclimatized to the laboratory environment for a week under 12-12 h light dark cycle. The animals were fed on standard pellet diet and water *ad libitum*. The animals were fasted 24 h before induction of diabetes [2-5 groups] by intraperitoneal (i.p) injection of 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 control group [1]. And diabetic group [5]. Received 10 ml distilled water. Animal in group 2 received VA (100 mg/kg) while animal in groups 3 and 4 received standard drugs Glibenclamide (2 mg/kg) and Metformin (500 mg/kg) were given 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].

$$\text{Percentage Blood Glucose Reduction} = \frac{1-Ff}{Fi} x100 \text{---------- (1)}$$

Where

$F_{f=}$ Final mean blood glucose, $F_{i=}$ Initial mean blood glucose

Formulation of soft lozenges

The lozenges were prepared using the formula shown in Table 1. The accurate weighed Polyethylene Glycol (PEG) was placed in 500 ml beaker and was gently heated directly on a heater to temperature of 70°. The weighed freeze dried aqueous extract of VA extract was triturated and added to the PEG solution. Homogenous mixture was obtained after thorough stirring for 30 min using constant temperature magnetic stirrer. The mixture was poured into calibrated lozenges mold and allowed to cool. The lozenges were stored in refrigerator [8].

Table 1: Formula for VA soft lozenges.

Drug/ excipients	Qty/1 Lozenge (mg)	Qty/4 in 1 Lozenge (mg)	Qty/12 (g)
VAALE (150 mg/kg) Rat Wt 140 g	21	84	1.008
PEG1000	1107	1107	12.690

Evaluation of lozenges containing freeze-dried VA leaf extract

The evaluation of lozenges containing freeze dried VA includes physical observation, weight uniformity test, specific gravity, disintegration test, thickness measurement, in-vitro drug release and physical stability Physical observation including colour, clarity, surface texture and appearance of all lozenges was carried out visually.

Weight uniformity test

Twenty lozenges of VA individual and combined were weighed. The average weight, the deviation, the percentage deviation of various lozenges were calculated as described in literature [13].

Specific gravity determination

Specific gravity was determined for VA lozenges using a 10 ml beaker containing 5 ml of water. A weighed VA lozenges was placed in the beaker and final volume noted. The change in the volume was taken as volume occupied by lozenges. The specific gravity was calculated as stated in equation [2].

$$\text{Specific Gravity} = \frac{(\text{Weight of the Lozenges})}{(\text{Volume of the Lozengez})} \quad \text{----------(2)}$$

Dissolution test of extract Lozenges

A 200 ml quantity of distilled water was heated to 37°C on a magnetic stirrer unit (Search-tech, China) set at about 50 rpm. A lozenges was added to the water and observation of extent of melting was recorded with time.

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

A 100 mg quantity of VA 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 VA 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 277 nm (λ max).

Dose-related anti diabetic response of aqueous VA leaf extract lozenges

Forty two rats of male sex weighing 120-150 g were randomly divided into 7 groups [1-7]. Of five rats per group and fasted for 14 h. Each rat in each group was intraperitonealy 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 aqueous VA lozenges accurately prepared with respect to weight of rat was orally administered at doses of 100, 150, 200 mg/kg for groups 2-4 respectively. The drug was administered by placing a quarter of lozenges under the tongue and closing the lips. Then standard glibenclamide (2 mg/kg) and Metformin (500 mg/kg) lozenges were administered to groups 5 and 6 respectively. The control group [1]. and diabetic group [7]. Received 2 ml/kg of normal saline orally. Blood glucose of the rats was measured at [0, 1, 2, 4 and 8] h. The percentage glucose reduction was calculated according equation 1.

Effect of combination freeze-dried aqueous VA and standard lozenges on STZ -induced diabetic rats

Forty two healthy male albino rats (100-150 g) were randomly selected and housed in ten 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 Streptozocin (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 of normal saline. Test groups 2-4 received lozenges of VA (150 mg/kg), VA and GLI (150:2 mg/kg), VA and MET (150:500 mg/kg) respectively. In groups 5 and 6, standard drug Glibenclamide (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 using equation 1.

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

Thirty rats of male sex weighing 120-150 g were randomly divided into groups coded [1-5]. Of six rats per group and fasted for 14 h. Each rat in each group was intraperitoneally 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 control 1) and diabetic group 5 received 2 ml/kg of normal saline orally. The VA lozenges accurately prepared were orally administered at doses of 150 mg /kg to group 2. Standard drugs Glibenclamide (2 mg/kg) and Metformin (500 mg/kg) lozenges were administered to group 3 and 4 respectively. The treatment of STZ induced diabetic rat was done for two weeks.

The feeding habit and volume of water taken were determined daily. At the end of treatment, the weight of rats and the blood glucose were measured.

Statistical analyses

The results generated from the various examination 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 lee tan 0,05 was considered significant.

Results

Herbal plants have been shown to possess anti-diabetic activity. Hence, in this study, lozenges solid dosage form containing freeze dried aqueous extract of VA were formulated and evaluated. The phytochemical analytical results of VA aqueous leaf extract are shown in Table 2. The extract contains flavonoids, saponins, tannin and alkaloids. The preliminary screenings of aqueous extract show that VA had 57 % blood glucose reductions. Freeze dried VA lonzenges had good appearance. Physical examination showed they are brown, clear and had smooth oily surface. The weight uniformity of VA lozenges was satisfactory. The percentage deviations of lozenges were within *pharmacopoeia* specification (non-deviated by ±10%). The specific gravity of VA and Metformin lozenges were 0.539 and 0.485 respectively. All lozenges dissolved after 5 min. In-vitro drug release study revealed that 100% of all drugs were released after 4.5 min as shown in Figure 1.

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

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

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

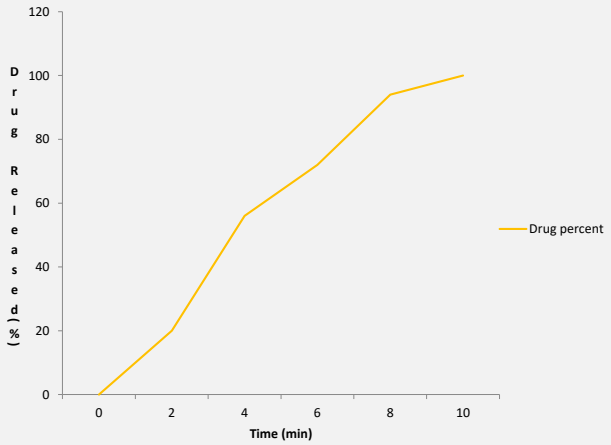

Figure 1: Drug release of VA lozenges.

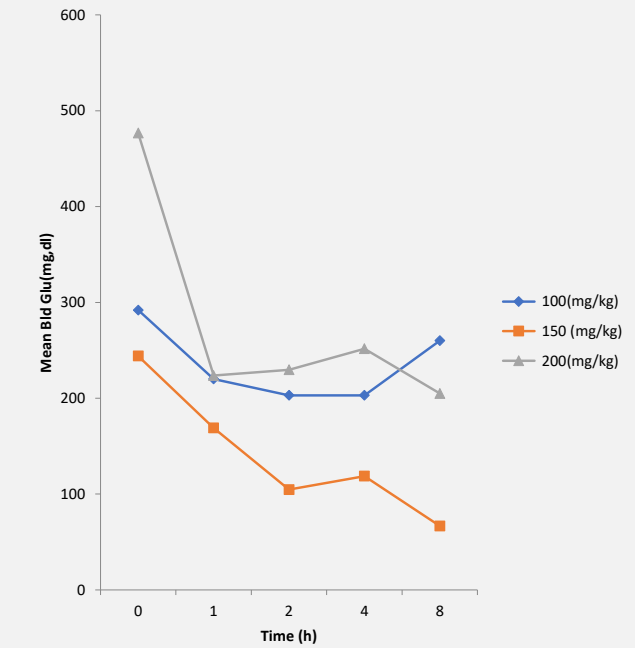

Figure 2: Blood glucose against time (h) for different concentrations of VAALE lozenges VA lozenges effect on blood glucose of Streptozotocin (STZ) - induced diabetic rats.

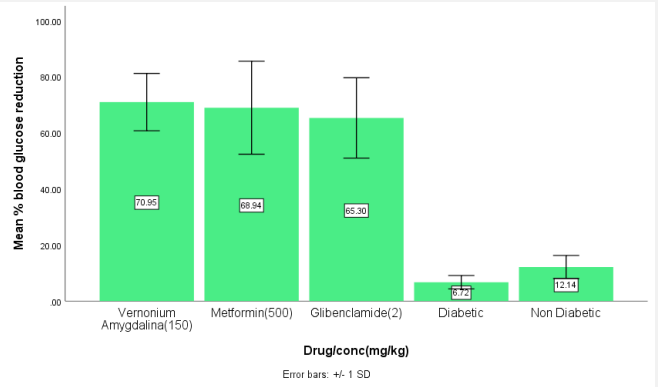

Figure 3: Comparative anti diabetic studies for various lozenges effect of combination of VA aqueous leaf extract and standard lozenges on blood glucose of diabetic rats.

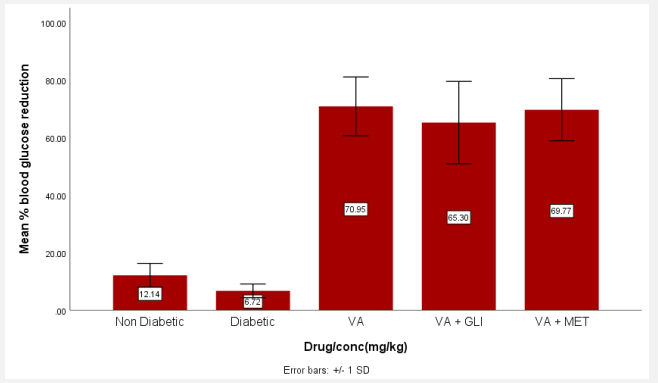

Figure 4: Percentage blood glucose reductions of various VA combined with standard effect of VA lozenges on diabetic rats on subacute treatment.

Physical examination of the VA lozenges/ showed no signs of discoloration, dryness, cracking and mottling mold growth after eight weeks of observation. The effect of dose-related anti diabetic response of aqueous leaf extract of VA lozenges.

The mean blood glucose-time graph of various concentrations of VA is presented in Figure 2. VA dose of 150 mg/kg concentration had maximal blood glucose reduction of 70.95%.The anti-diabetic effect of VA aqueous extract lozenges on STZ induced diabetic rats is shown in Figure 3. VA lozenges had the highest percentage blood glucose reduction (70.95 %). There was no significant difference between VA and standard drugs (Met and GLI) troches P>0.05. The percentage blood glucose reductions of VA combinations with standard lozenges are represented in Figure 4. There was no significant difference in percentage blood glucose reduction between VA individual and the combination with standard lozenges P>0.05.

Treatment of diabetic rats with VA lozenges was carried out for two weeks. The feeding habit during the course of treatment is summarized in Table 3. The changes in weights and blood glucose levels at the end of therapy are summarized in Table 4. Among the treatment groups, VA treated animals took food fraction per day which was not different from other standard treatment groups. 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. Diabetic rat had a weight loss. VA treated rats had significant increase in weight compared to the diabetic control group. There was a great improvement in blood glucose reductions at the end of 14 days treatment for therapy for all treatment group. As shown in Table 4.

Table 3: Feeding habit of the treated rats during the course of treatment.

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
Cons	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
VA	33.5	68.8	43.8	75	35	50	35	92.8	20	66.7	33.3	66.7	46.7	100	51	100	67.7	66.7	67.7	66.7	50	66.7	51.7	66.7	53	66.7	53.7	75
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 and final blood glucose after 14 days treatment.

Drug (mg/kg)	Initial Day Weight (g)	Final Day Weight (g)	Change in Weight (g)	Final Blood Glucose (mg/dl)
VA(150)	107.2	111.67	7.8	71.33
GLI(2) **MET(500)**	111.67 113.67	116.33 122.93	4.67 9.27	84 74
Diabetic	121.67	101.47	-20.33	239.33
Normal	115	123.57	8.57	70.33

*Mean (SD).

Discussion

The preliminary screenings of aqueous extract show that VA had 57% blood glucose reductions. The result of preliminary screening study indicates that VA had anti-hyperglycemic effect. The dose for the effect of aqueous extract on diabetic animals screened was based on previous report [3]. VA lozenges had a good appearance with a clear brown, smooth surface. Weight uniformity of all the lozenges passed the Pharmacopoeia specifications as non-deviated by 10% [13]. This depict that the lozenges had content uniformity. The good physical results of lozenges after eight weeks of storage may be attributed to proper selection of Polyethylene Glycol (PEG) 1000 as the base and optimized formula. It may be also suggested that no incompatibility existed between VA aqueous extract and base PEG 1000. VA lozenges dissolved after 5 min and released 100% drug showing that the VA drug will be bio-available. The drug content of VA lozenges met pharmacopoeia's specifications as it was in the range of 90-110% which is the most theoretical drug content range requirements for most drugs according to British Pharmacopeia specifications [14].

VA lozenges had the highest percentage blood glucose reduction (70.95%) on diabetic rat when compared to the standard. The marked reduction in blood glucose could be attributed to phytochemical composition of VA that may regenerate β-cells and other extra pancreatic mechanism such as insulin mimetic action and oxidative stress attenuation [15]. There was no significant difference in percentage blood glucose reduction between VA individual lozenges and the combination with standard (GLI and MET) lozenges P>0.05. There is no advantage of combination with standard over the individual lozenges. Therefore, preparation of individual VA would benefit the users and producers and will also be cheaper. The results of treatment of diabetic rats with VA lozenges for two weeks revealed significant increase in weight compared to the normal control group. Diabetic rat had a weight loss which would be attributed to loss in muscles and adipose tissues due to increased metabolic rate and glycosuria [5,16]. Weight gain in VA lozenges/troches treated rats resulted from the tissues accessing the glucose both to supply energy and to build tissue materials required for growth. This result is consistent with a study on effect of purified fraction of VA on diabetic rat [17]. Moreover, the final fasting blood glucose levels of VA treatment groups after 14 days of therapy were in comparison with normal control. The phytochemical constituents of VA namely flavonoids, saponin and tannin [16,18] had contributed to lowering effect of diabetic rats blood sugar. It was also explained that VA regenerated damaged β cells by streptozotocin therefore increasing secretion of C-pepetides

and insulin [19]. This in-turn will cause significant reduction in blood glucose and weight gain.

Conclusion

Lozenges containing VA aqueous leaf extract (150 mg/kg) was prepared and they passed pharmacopeia requirements. It had significant anti-diabetic activity. The combination of VA with standard drugs had no additional significant anti-diabetic effect. This work has shown that inelegant, bitter, non-specific dosing liquid extract of VA can be transformed into lozenges solid dosage forms. The product can be a personalized medicine for very sick diabetic patients that are unable to swallow VA capsules/tablets.s.

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