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Formulation and Evaluation of Tablets from Antidiabetic Alkaloid Glycosin

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Mangroves derived alkaloid on medications of diabetes mellitus are insufficient. The oral route is widely preferred convenience way of drug administration. Therefore, the present study was aimed to formulate and evaluate tablet from the anti-diabetic alkaloid glycosin from *Rhizophora apiculata*. Powder form of glycosin (10 g) mixed with suitable active ingredient and excipients to formulate tablets by punching method. The starch, aerosol colloidal silicon dioxide, gelatin, magnesium stearate selected as diluents, disintegrant, binding agent and lubricant selected for glycosin tablet formulation. Tablets determined for mass, hardness, disintegration, friability, dissolution and stability. Each glycosin tablet contain 50 mg of the active ingredients. The weight, diameter and thickness of each glycosin tablet were 50 mg and 10 mm, the variation in thickness was 5% deviation limit. Glycosin tablets showed potential hardness (3.5-4.5 kg cm^{-2}) which facilitates its rapid disintegration within 35.22-36.23 min. The percentage of difference in friability was 0.351% indicated that the of glycosin tablets were mechanically stable. The overall dissolution patterns of the six glycosin tablet were characterized by 50% rapid release in the first 15 min followed by 90% at 75 min. The tablet showed potential stability when stored at different storage conditions for 40 days. The formulated glycosin tablet would be great potential in the treatment of diabetes mellitus.

Key words: Alkaloid, diabetes mellitus, mangrove, tablet, stability

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INTRODUCTION

Type 2 diabetes mellitus is a heterogeneous, multifactorial, polygenic disease usually characterized by variable degrees of insulin resistance, impaired insulin secretion and increased glucose production. Distinct genetic and metabolic defects in insulin action and/or secretion give rest to the common phenotype of hyperglycemia in T2DM (Gardner and Shoback, 2011). There are two major classes of drugs used for the treatment of type 1 diabetes is sulfonylureas which act mainly by increasing endogenous insulin secretion while biguanides act chiefly by decreasing hepatic gluconeogenesis and increasing peripheral utilisation of glucose. Oral treatment of T2DM in non-obese patients is usually begun with a sulfonylurea. There is evidence that the use of low-dose of sulfonylurea therapy in patients with diagnosed T2DM but near-normoglycemia (Simo and Hernandez, 2002). Metformin is as effective as the sulfonylurea in terms of blood glucose control and is less likely to cause hypoglycemia but has a rare tendency to cause lactic acidosis in patients with renal impairment, in whom it should not be used (Simo and Hernandez, 2002). Therefore, researchers focused their interest towards secondary metabolites of medicinal plants including alkaloids to control hyperglycemic conditions. The oral route is widely preferred convenience way for people for drug administration. Today tablets are the most common pharmaceutical dosage forms. Those are popular for the following reasons such as convenient to handle because they are portable and easy to be administered, the cost of manufacturing, packaging and shipping is relatively low from the manufacturers point of view. Formulation enables high dose of active substances to design smaller than capsule containing granules or powder, as well as granules or powder themselves and solid dosage forms such as tablets, capsules shows a much higher physical and chemical stability compared with liquid formulations. Disintegrant is used to aid the disintegration of tablets. Determination of the critical concentration of disintegrant is an important parameter for a tablet formulation design, especially for low water soluble drugs. Therefore, several research groups reported the critical concentration of disintegrant that is required to achieve the minimum disintegration time (Ringard and Guyot-Hermann, 1988).

For example, pyrrolidine alkaloids (Radicamines A and B) from *Lobelia chinensis*; quinolizidine alkaloids (Javaberine A, hexa-acetate, lupanine) from *Talinum paniculatum* and *Lupinus perennis*; polyhydroxylated alkaloids (2-hydroxymethyl-3,4-dihydroxy-pyrrolidine-N-propionamide) from the root bark of *Morus alba* were act as a potential alpha glucosidase inhibitors, enhancer for glucose-induced insulin release from isolated rat islet cells and useful diet supplement for prevention of diabetes (Yuzo *et al*., 2003; Asano *et al*., 2001). *Rhizophora apiculata* used as traditional medicine in the coastal village of Tamil Nadu, especially for the fisher women community (Kaliamurthi *et al*., 2014). There are 15 alkaloids identified from *R. apiculata*. The antidiabetic effect of major glycosin alkaloid was evaluated and filed a patent (Gurudeeban, 2013). Moreover, the DNA barcode of this medicinal plant deposited in National Center for Biotechnological Information, USA and Barcode of Life (BOLD) database for commercialization of the product (Gurudeeban *et al*., 2015). In this connection, the present study aimed to formulate antidiabetic tablets from glycosin alkaloid and characterized their physiochemical parameters under *in vitro* condition.

MATERIALS AND METHODS

Chemicals: Aerosil, starch, gelatine, magnesium stearate, sodium lauryl sulphate, hydrated magnesium silicate, methylparaben sodium, propylparaben sodium procured from Sigma-Aldrich (Mumbai, India). The solvents used in the experiment were of analytical grade.

Source of drug: Glycosin is a quinazoline class of alkaloid with the molecular weight of 250.3 g mol^{-1}. The 100% HPLC purified form of glycosin was used to formulate tablets (Fig. 1).

Determination of the amount of active ingredient in the tablet: The amount of the active ingredient to be incorporated, into the tablet was based on the yield of glycosin alkaloid obtained from the *R. apiculata*. Two grams of glycosin powder calculated to be equivalent to the 8 g of fresh leaves that estimated to produce one 150 mL cup of infusion. The filtrate of the 2 g/150 mL infusion produced 100 mg of dried glycosin powder, a yield that remained the same on scale-up. Based on the above calculations and findings, it consequently decided that 100 mg of glycosin considered the amount of a single dose. For experimental convenience, this dose divided into two tablets, each tablet containing 50 mg of the active ingredient.

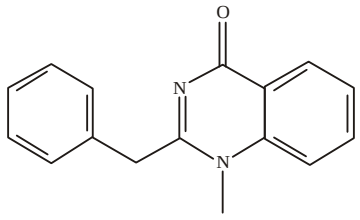

Fig. 1: Structure of glycosin

Selection of tablet punching method: While selecting the tablet formulation methods, compressible characteristics of the drug are considered. For drugs which are poorly compressible, have moderate to high-dose the most obvious and direct approach would be to follow wet granulation methodology. For drugs with low to moderate doses, direct compression technique offers various advantages to the pharmaceutical formulator in terms of economy, because less number of processing step, persons and time is required, stability, because product is not required to expose to a moisture and heat, performance and since tablets will directly disintegrate gives higher dissolution. The present study, the direct compression technique employed to prepare conventional dosage form of glycosin tablets (Chandira *et al*., 2012).

Selection of excipients and formulations: The method of tablet manufacture and the physicochemical characteristics of the active ingredient were the main criteria used in the selection of the excipients for the formulation. In turn, their suitability for compression was the primary requirement considered when selecting the excipients. The density and flowability of glycosin influenced the selection of the excipients. Thus, aerosil was the selected excipients for the tablet formulation. The selected excipients considered and used in the experimental study (Chandira *et al*., 2012).

Diluents: The diluents most widely used in tablet formulations are lactose, starch, microcrystalline cellulose, dibasic calcium phosphate and mannitol. Starch is used as diluent in the formulation of glycosin tablet (Shah and Arambulo, 1974).

Disintegrants: Aerosil colloidal silicon dioxide was selected as the disintegrant its ability to absorb approximately 9 times more moisture than starch at a several times slower rate was considered to be a significant factor (Marshall, 1979).

Binders: Gelatine is a good binding agent, co-diluent as well as a disintegrant. It has good flowability, appropriate particle size and is well suited for direct compression (Patel *et al*., 2012).

Antifriction agents: Magnesium stearate and talc were used as lubricant and glidant, respectively. Sodium lauryl sulphate is a soluble lubricant that acts as a surfactant and facilitates the rapid disintegration and enhanced dispersion of the tablet. Lubricants act by interposing an intermediate layer between the tablet constituents and the die wall to reduce die wall friction when the tablet surface moves relative to the die upon compression and ejection. Hydrophobic lubricants like magnesium stearate also form a film like layer over the tablet surface that gives shine and elegance to the tablet. Because of magnesium stearate's hydrophobic nature, its consequent effects on the release of the medicament and the significant reduction of mechanical strength of tablet, the concentration of magnesium stearate was kept low in the formulations elaborated in this study (Bi *et al*., 1999).

Table 1: Formula for the processing of trial tablets

Ingredients	Working formula (for 50 tablets)
Sample drug	10 g
Gelatin	60 g
Hydrated magnesium silicate	120 g
Magnesium stearate	120 g
Aerosol	120 g
Starch	1.2 kg
Methyl paraben sodium	0.022 g
Propyl paraben sodium	0.004 g

Formulation of tablets: There is no information on the manufacture of mangrove derived alkaloid based tablets in the literature. Thus, the development of the formulation for the present study was based on a trial method basis (Table 1). The development of the formulation proceeded in a stepwise manner to bring about a final formula that contained 50 mg of alkaloid glycosin and complied with the minimum quality control requirements for mass uniformity, hardness, size and shape. The tablets were prepared by wet granulation method. All the ingredients were weighed and triturated and passed through sieve No. 8 separately. The drug, half amount of starch, methylparaben sodium and propylparaben sodium were mixed and to this gelatin was added to form a coherent mass. The mass was passed through sieve No. 16 to form granules and the granules were dried at 50°C for 15 min in a hot air oven. To the dried granules rest of the starch and magnesium stearate, talc and aerosol were added and blended with a spatula. Finally, the granules were subjected to compression by single punching machine to obtain tablets.

Evaluation of the glycosin tablets

Equality of mass: The manufactured tablets were tested for mass uniformity using the method described in 1999 Indian Pharmacopoeia. Twenty tablets were randomly chosen from each trial batch, each tablet was individually weighed and the average weight per batch of tablets was calculated. From this, the deviation in the individual tablet weights and the standard deviation were also calculated (Babu *et al*., 2003).

Standardization of size and shape: The diameter and thickness of the manufactured tablet are indicators of the size and shape of the tablets. The size and shape of the tablets produced depended not only on the volume and weight of the fill mass but also on the diameter of the die and the pressure applied to the fill on compaction. In

order to produce tablets of uniform thickness during the production of each formulation, care was taken to employ the same volume of the fill and the same compaction pressure. It should be noted that the pressure applied to the tablet press not only affected the thickness of the tablet but also its hardness. In the present study, 20 tablets from each manufactured batch were randomly checked for diameter and thickness (Babu *et al*., 2003).

Hardness test: The hardness test is not a standard test, it was included in the quality control procedures for tablets. Since the hardness of the tablet plays an important role in its disintegration rate. Pfizer hardness tester was used to measure the hardness. Tablet was placed between spindle and anvil of the tester and the calibrated scale adjusted to zero then applied a diametric compression force on the tablet and the position on the calibrated scale at which the tablet broke was recorded in kg units. A mean value is taken to check for their hardness (Bi *et al*., 1999).

Disintegration test: One tablet was introduced into each tube and a disc was added to each tube. Suspended the assembly in a beaker containing the water. The volume of the water, should be such that the wire mesh at its highest point is at least 25 mm below the surface liquid and its lowest point is at least 25 mm above the bottom of the beaker and operates the apparatus and note the time of disintegration (Bi *et al*., 1999).

Friability test: The friability test was to determine the tablets ability to retain its physical properties against the effects of friction, shock or vibration during the various processing steps (Bi *et al*., 1999). Ten tablets were randomly selected from each trial batch, dusted with a small brush to remove any adhering powder and weighed (W1). The tablets were then placed in the friability which was operated at 20×g for 4 min. Thereafter the tablets were removed from the apparatus, again dusted with a small brush and weighed (W2). The percentage of weight loss was determined using the following equation:

$$\text{Percentage of weight loss} = \frac{W1-W2}{W1}\times 100$$

Dissolution test: The dissolution profile of the tablets was determined by using the specified procedure for dissolution test and the samples analysed by using a UV-visible spectrophotometer. Six single tablets were prepared by dissolving the drug in phosphate buffer pH 7.4. The mixture was stirred with the paddle rotating at 50 rpm. Five milliliter sample solutions were withdrawn, with a syringe, at 15 min intervals up to 90 min. Each sample withdrawn was immediately replaced with an equal volume of phosphate buffer. The collected samples were passed through a membrane filter and the absorbance of the filtrate determined in the spectrophotometer at the wavelength of maximum absorption. To determine the wavelength of maximum absorption for the glycosin, a dilute solution (0.01% w/v) of the active ingredient was prepared and scanned for its absorption spectrum over the wavelength ranging from 260-560 nm. The concentration of dissolved glycosin tablets in each sample was determined from a standard curve of the UV absorbencies of samples of known concentrations i.e., 10, 20, 30, 40 and 50 μg mL^{-1} of the active ingredient. After determining the concentration of the samples from the standard curve, the percentage drug release was calculated and plotted versus time to obtain the dissolution profile was analysed using Graph Pad Instat software to obtain the dissolution curves (Bi *et al*., 1999).

Stability studies: The manufactured tablets were also tested for changes in physicochemical properties after various samples of tablets were stored under different storage conditions for 40 days. Some tablets were stored at 40°C and 75% relative humidity, some at 25°C and ordinary room level relative humidity and some at 5°C and 0% relative humidity. The physicochemical changes i.e., changes in size, shape, colour, odour, hardness, disintegration time, content uniformity and moisture content of the dosage form were determined every 10 days over a 40 day period (Bi *et al*., 1999).

RESULTS

Uniformity, size and shape of glycosin tablets: The weight, diameter and thickness of the 20 tablets were 50 mg and 10 mm. The general requirement of Indian Pharmacopoeia for mass uniformity is that no more than two tablets should deviate from the average weight by more than ±5%. The deviation in a mass of the glycosin tablets manufactured within these limits of the Pharmacopoeia. The average diameters of the tablets were 10 mm and the variation in thickness within the 5% deviation limit (Fig. 2).

Hardness studies of glycosin tablets: There are no specifications set in Pharmacopoeia for the hardness of tablets, but generally, a hardness of 55 N is considered for conventional tablets. Table 2 indicated that the glycosin tablets manufactured in this study had a wide range of hardness with the average value being 4.0 kg cm^{-2} which may be considered to be very high. The hardness values also varied greatly, from 3.5, 4.0, 4.5 kg cm^{-2} and the physical properties of the glycosin was probably primarily responsible for this hardness. It could thus be concluded that the tablets exhibited very high hardness values and this could very possibly impact on the disintegration and dissolution of the tablets.

Fig. 2: Formulated glycosin tablets

Table 2: Hardness test of formulated glycosin tablets

Hardness of each tablet (kg cm^{-2})	Average hardness (kg cm^{-2})
3.5	4.0
4.0	
4.0	

Table 3: Disintegration test of formulated glycosin tablets

Disintegration time	Average disintegration time
35 min 22 sec	36 min 22 sec
36 min 21 sec	
36 min 23 sec	

Table 4: Standard curve of formulated glycosin tablets

Concentration (μg mL^{-1})	Absorbance (nm)
10	0.182
20	0.361
30	0.592
40	0.723
50	0.904

Table 5: Dissolution study of formulated glycosin tablets

Time (min)	Absorbance (nm)	Concentration (mg mL^{-1})	Amount in 900 mL	Drug release (%)
15	0.009	0.50	0.45	0.9
30	0.016	0.88	0.80	1.6
45	0.027	1.50	1.35	2.7
60	0.083	4.60	4.15	8.3
75	0.194	10.70	9.70	19.4
90	0.205	11.30	10.25	20.5

Friability studies of glycosin tablets: The results for the friability test were as follows: Initial, end and difference in weight of 10 tablets after the test is 4.556, 4.540 and 0.016 g. Percentage difference in weight (friability) = 0.351% i.e., there was less than 1% change in weight. The general specification is that a change in weight of less than 1% is required to pass the friability test. The friability test of glycosin tablets, indicating that the tablets would withstand the physical rigors expected in handling chipping, cracking and breaking of the tablets would not occur.

Disintegration studies of glycosin tablets: The results indicating the disintegration characteristics of the tablets are shown in Table 3 and 4. The disintegrated time of glycosin tablets within 35.22-36.23 min. Despite the high hardness values, the tablets thus still showed acceptable disintegration characteristics. The tablets appeared to disintegrate by dissolving in the medium, rather than through a process of breaking up and releasing the particles of the tablets into the disintegration medium. As water penetrates into a tablet, it generally swells and finally breaks up. In tablets that dissolve instead of breaking up, it normally means water penetration of the tablet mass was poor. Possibly the magnesium stearate in the formulation reduced the wetting of the tablet material with the disintegration medium. Nevertheless, it seemed that the extreme hardness of the tablets did not impact negatively on their disintegration.

Dissolution studies of glycosin tablets: A wavelength scan of a 0.01% solution of the glycosin indicated that the wavelength of the maximum absorption was 282 nm. The 282 nm absorbances of various concentrations of the glycosin in phosphate buffer were thus measured and the results obtained are given in Table 5. Using these values the amounts of tablets dissolved after various times in the dissolution medium were calculated and the percent (%) drug released at the times estimated. The overall dissolution patterns of the 6 tablets were characterized by the rapid release of almost 50% in the first 15 min and increasingly less thereafter. After 75 min, more than 90% of tablet contents had been released. All these tablets passed the dissolution test requirements.

Stability studies of glycosin tablets: The various physicochemical properties of the formulated glycosin tablets were monitored while they were stored in 3 different sets of the condition. The tablets stored at site A had lost their physical properties after 10 days of beginning of the study. The 50 mg tablets stored under the three stability conditions showed an average increase of glycosin levels at the end of storage compared to the initial strength of glycosin in tablets.

DISCUSSION

Diabetes Mellitus (DM) is a serious and growing health problem worldwide and is associated with severe acute and chronic complications that negatively influence both the quality of life and survival of affected individuals. The development of glycosin tablets from the mangrove *R. apiculata* is undertaken in this study was an attempt to validate the traditional usage. There are studies reported formulation of *Luffa acutangula* and *Madhuca longifolia* showed potential antidiabetic effect on diabetic rats. Phytomedicines offer a greater advantage over synthetic drugs as they are cheaper and show no side effects (Singh *et al*., 2014).

Five isoquinoline alkaloids, berberine chloride, berberine sulfate, berberine iodide, palmatine sulfate and palmatine chloride had been isolated from the root of *Coptis japonica* and their inhibitory activity against lens aldose reductase was measured (Lee, 2002). Recently, 1,4-dideoxy-1,4-imino-D-arabinitol alkaloid found to be a potent inhibitor of glycogen phosphorylase and commercially available for the treatment of diabetes in several countries (Yamashita *et al*., 2002). These literatures supports the present results in alkaloids based formulations are effective antidiabetic agents.

Starch contains 12-14% of moisture and is believed to stabilize hygroscopic drugs by balancing the moisture level in the hygroscopic substance(s) and itself (Banker and Anderson, 1987). In the present study, the glycosin tablets however became soft, damp and changed their colour to light greenish yellow within a few hours of formulation. Moreover, the presence of magnesium stearate might have had adverse effects on the performance of Starch (Marshall, 1979), providing another reason for the exclusion of the starch. Other diluent options, starch which is useful for direct compression, were however not considered since they may be very hygroscopic under conditions of above 65% relative humidity (Banker and Anderson, 1987). The selection of the direct compression method for the manufacture of the tablets was based on the physical properties of the active ingredient and this also was not unusual. However, in this case, the formulation of glycosin tablets from *R. apiculata* is required special conditions to obtain the desirable tablets. Several excipients were tried and the selection of those suitable for the dosage form was arrived at by using the "Trial and Error" method. The tablets were made and evaluated, in sequential order and depending on the results obtained, the proportions of certain excipients were increased or decreased or some excipients actually replaced with new excipients. Through the above process, the following decisions were made with respect to the excipients (Chandira *et al*., 2012).

CONCLUSION

The formulated glycosin tablets evaluated for physical parameters and standardize as per pharmacopoeial standards. Moreover, glycosin tablets will be as promising antidiabetic drug in near future. The clinical trails are in progress.

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