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Standardization of marketed formulation Glucomap for antidiabetic action

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Abstract

The primary objective of the current investigation was to standardize the formulation Glucomap Tablet, marketed as an effective treatment for management of diabetes. The procured tablets of GMT were brownish green in color, with no odor and a bitter taste. They were available as rough textured tablets with no coating, stored free from moisture. GMT exhibited 8.3% total ash with 2.05% acid insoluble ash and 5.75% water soluble ash. The water soluble and alcohol soluble extractives were 1.14% and 1.22% respectively suggesting the formulation to be suitable for human use. The results of preliminary qualitative phytochemical screening revealed that the classes of phytochemicals were present in GMT. TLC analysis of GMT was done using charantin as the marker using methanol: benzene (2:8) as the solvent system. Charantin appeared at Rf value of 0.56 on the TLC plate. The quantitation of the charantin was done by HPLC method and it was found that GMT contained 8.6 mg charantin per 500 mg of GMT (1.72 %). The antidiabetic potential of the GMT tablet was determined by extracting the tablets with ethanol and administering at a dose of 200mg/kg to alloxan induced diabetic rats. GMTEE was able to decrease blood sugar by 23.62% while the standard drug glibenclamide could reduce it by 28.12% on the 15th day.

Keywords: Standardization, herbal, glucomap, charantin, antidiabetic, WHO

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Introduction

Ayurveda is a respected medicinal framework that started in India a huge number of years ago. The term "Ayurveda" in this manner signifies 'the information on life' or 'the study of life'. Medicinal plants, for a few centuries, have been broadly utilized as an essential wellspring of counteraction and control of domesticated animal infections. Herbal medicinal plants are an essential segment of research advancements in the pharmaceutical business. The World Health Organization (WHO) had given a short convention for standardization of homegrown medications. Standardization is exceptionally conspicuous to guarantee that each completed item that enters showcase liberated from corruption. Nowadays, there is have to standardize Ayurvedic formulation in uniform quality.

Glucomap tablet is manufactured and marketed by Maharishi Ayurveda for diabetes management (maharishiayurvedaindia.com, 2021). It is a rational combination of herbal hypoglycaemias known since ages and duly accredited by laboratory and clinical studies. This Ayurvedic formulation has both pancreatic as well as extra pancreatic action and is highly effective for people with pre-diabetic condition. Glucomap is claimed to stimulate beta cells of the pancreas, increase peripheral utilization of glucose and arrests glycogenolysis. It is also claimed to protect cardiovascular and nerve tissues from degenerative changes, protects the liver and strengthens its function.

The primary objective of this work was to standardize Glucomap Tablet with respect to its physicochemical properties, organoleptic properties, marker quantitation and evaluation of its antidiabetic action.

Material and Methods

Glucomap Tablet (GMT) was purchased from the online store of Maharishi ayurveda (India) Pvt Ltd. Charantin was used as the marker compound and was extracted as per reported procedure. All chemicals and reagents used were for AR grade and purchased from Oxford Fine Chemicals, Mumbai. Experimental animal were procured from approved local breeders.

Organoleptic Standardization of GMT

Organoleptic properties are the aspects of food or other substances as experienced by the senses, including taste, sight, smell, and touch, in cases where dryness, moisture, and stale-fresh factors are to be considered (Anonymous, 1998).

Physicochemical Standardization of GMT

Physiochemical studies such as water soluble extractives, alcohol soluble extractives, ether soluble extractives, hydro alcoholic soluble extractives, total ash, water soluble ash, acid insoluble ash, were carried out as per the WHO guide lines. The tablets were powdered using a clean and dry mortar and pestle for determination of the physicochemical parameters.

Preliminary Phytochemical Screening of GMT (Mehta et al., 2017)

Alkaloids

The testing for presence of alkaloids was done by dissolving GMT in 5 mL of 1% HCl solution. This solution was then subjected to for alkaloids.

Mayer's test To a few mL of GMT solution, two drops of Mayer's reagent was added along the sides of test tube. The formation of a cream colored precipitate was taken as indicator of a positive response to the test.

Glycosides

The test for presence of glycosides (deoxy sugar) was performed according to **Keller-Killiani's** method. GMT is *e*xtracted with chloroform and evaporated to dryness. To this residue was added 0.4 mL of glacial acetic acid containing a trace amount of ferric chloride. The solution was transferred to a test tube and 0.5 mL of concentrated sulphuric acid is added along the wall of the test tube. Brown ring formed at the interface may indicate the presence of deoxysugar cardenolides. A violet ring may appear just below the brown ring, while in the acetic acid layer, a greenish ring may also form gradually throughout the layer, if the cardiac glycosides are present.

Saponins

0.1 g of GMT was boiled in 1 mL distilled water and filtered. To the filtrate was added 3 ml distilled water, shaken vigorously and heated. The sample was observed for the persistence appearance of foam lasting for at least 15 min was taken as confirmation for the presence of saponins.

Tannins and phenolic compounds

To the sample of GMT was added a freshly prepared solution of ferric chloride. Development of blue-green color is taken as indication for the presence of tannins and phenolics.

Alkaline reagent test was performed for confirming the presence of tannins. Test solution of GMT is treated with sodium hydroxide solution. The formation of yellow or red colored precipitate is an indication of the presence of tannins in the sample.

Flavonoids

To the test solution of GMT, a mixture of zinc dust and concentrated hydrochloric acid was added. If the colour of the solution changes to red, it is taken as a confirmation for the presence of flavonoids.

Proteins and Amino acids

0.1 g of GMT was dispersed in water and was boiled with 0.2% solution of ninhydrin. Development of violet colour in the solutions is an indicator for the presence of amino acids and proteins in the sample.

Sterols and Triterpenoids

Libermann-Burchard test was performed to detect the presence of steroids in the formulation. GMT was treated with few drops of acetic anhydride, boiled and cooled. Concentrated sulphuric acid was

added from the sides of the test tube. Change in color from violet to blue or green indicates the presence of steroids.

Salkowski test was done to confirm the presence or absence of triterpenoids. GMT was dissolved in chloroform and a few drops of concentrated sulphuric acid were added to it. The mixture was shaken well and allowed to stand for some time to separate into layers. The formation of greyish colour indicates the presence of terpenes/terpenoids.

Extraction of charantin from Momordica charantia (Patel et al., 2010)

Momordica Charantia extract powder containing 10% charantin was purchased from E commerce store (amazon). The powder was percolated successively with petroleum ether (60-80°) and 80% ethanol. The petroleum ether extract was rejected and the ethanolic extract was concentrated in vacuum, then suspended in 95% ethanol and rendered alkaline with KOH to around pH 10. After 48 hours, the suspension was diluted with water and extracted with ether. The ether extract was washed with water, 5% Hydrochloric acid, again with water, and dried over anhydrous sodium sulphate. The ether was distilled off and the residue recrystallized several times from 95% ethanol to obtain charantin.

The separated charantin was tested using Libermann-Burchard method to confirm presence of steroidal structure.

TLC analysis of GMT and charantin standard

TLC- method was used by employing Precoated TLC Plate (Silica gel 60 F_{254}) for the standardization of GMT. Charantin and GMT were separately dissolved in ethanol and TLC was performed using methanol:benzene (2:8) as the solvent system for TLC. Concentrated sulfuric acid was used as the detecting agent for spot of charantin in both the samples.

Quantitative estimation of charantin in GMT (Zold et al., 2019)

Charantin in the GMT was quantified by a HPLC method which involved using a C18 column, UV detector and detection wavelength of 278 nm at a flow rate of 2.5 mL/min for the mobile phase comprising of acetonitrile, dichloromethane (40:60, v/v) and 0.1% phosphoric acid. The total duration of run was 20 min. Charantin standard solutions were prepared in various concentrations of 10, 20, 30, 40, 50 and 60 µg/mL by diluting the stock solution prepared in ethanol.

Evaluation of antidiabetic activity of GMT

Animals

Healthy Wistar rats of either sex, weighing 180-250g were used for the study and housed in polypropylene cages. The animals were housed in cages during the course of experimental period and maintained at 12 day and night schedule with a temperature [23 ± 2°C] maintained as standard experimental condition. The animals were fed with standard rodent pellet diet and

water *ad libitum*. The animals were fasted 12 hours before the experiment with free access to only water.

Induction of experimental diabetes

For induction of diabetes, animals were subjected to overnight fast (free access to water) for 12 hours to make them additionally susceptible to developing diabetes. Diabetes was induced in the test animals by intraperitoneally administrating alloxan monohydrate (150 mg/kg body weight) solubilized in normal saline. After 72 h mice with blood glucose range of 200 to 350 mg/dl were used for study.

Experimental Setup

Animals were categorized into seven groups, each consisting of six rats. Standard pellet diet and water *ad libitum* was provided to the animals.

Group I: Normal healthy rats administered only vehicle (0.5% Tween 80)

Group II: Diabetic control (Alloxan 150 mg/kg)

Group III: Diabetic rats of this group were administered with glibenclamide (10 mg/kg) from 6th day after first administration of alloxan

Group IV: Diabetic rats of this group were administered with GMT ethanolic extract (GMTEE 200 mg/kg) from 6th day after first administration of alloxan

Oral glucose tolerance test (Chandana et al., 2015)

Prior to initiation of the experimental procedure, the rats were fed with a bolus of 2g/kg dose of glucose and the level of glucose in blood was estimated at 0, 30, 60 and 120 seconds after administration of glucose using glucometer.

Evaluation of antidiabetic activity

The antidiabetic activity of GMT was determined by measuring the blood glucose levels on 1st, 10th and 15th day of administering the extract to the diabetic rats. The decline in glucose level was taken as the indicator for glucose ameliorating potential of the leaf extracts.

Results and Discussion

Organoleptic and physicochemical standardization of GMT

The GMT sample was brownish-green in color, with a bitter taste and rough texture and no odor. The results of water soluble extractives, alcohol soluble extractives, ether soluble extractives, hydro alcoholic soluble extractives, total ash, water soluble ash, acid insoluble ash are presented in table 1.

Table 1 Physicochemical properties of GMT

Parameter	Weight of Sample (g)	Weight of ash/extractive (g)	% Value
Total Ash	2	0.166	8.3
Acid insoluble Ash	2	0.041	2.05
Water soluble Ash	2	0.115	5.75
Water soluble Extractives	5	0.057	1.14
Alcohol soluble Extractives	5	0.061	1.22

Total ash value of is an indication of the amount of minerals and earthy materials present in the formulations. GMT exhibited 8.1% total ash with 2.35% acid insoluble ash and 4.95% water soluble ash. The water soluble and alcohol soluble extractives were 1.36% and 1.3% respectively suggesting the formulation to be suitable for human use.

Qualitative phytochemical screening

The powder of GMT was subjected to various chemical tests for preliminary screening of the class of phytoconstituents present in them. The result is presented in table 2.

Table 2 Phytochemical screening of GMT

Phytochemical Tested	Observation	Inference
Alkaloid	Cream precipitate formation in Mayer's Test	Present
Glycoside	Greenish color in acetic acid layer in Keller-Killiani Test	Present
Saponin	Frothing Formation	Present
Tannins	Yellow color precipitate in Alkaline Reagent Test	Present
Phenolics	Bluish green color in Ferric chloride Test	Present
Flavonoids	Red color formation in Zinc reduction Test	Present
Proteins and Amino acids	No color formation in Ninhydrin Test	Present
Sterols	Green Color in Burchard Test	Present
Triterpenoids	Grey color in Salkowski Test	Present

As it can be seen from the results all the classes of phytochemicals were present in GMT. The multiple plant extracts and parts used for formulation of the tablets might be responsible for the presence of all types of phytoconstituents in them. The presence of these multiple phytochemicals could easily be linked to the claim of treatment of several conditions associated with diabetes by the manufacturer.

TLC Analysis of GMT and Charantin

Charantin was extracted from *Momordica Charantia* powder using the reported method and the yield was found to be 0.87% by weight. The obtained product responded positive to Lieberman Burchard test confirming the steroidal structure.

TLC analysis of GMT was done using charantin as the marker using methanol: benzene (2:8) as the solvent system. The spots were visualized using concentrated sulfuric acid. UV Chamber as also used for spotting the other components of GMT. Charantin appeared at Rf value of 0.56 on the TLC plate.

Quantitation of charantin in GMT

Charantin was eluted using HPLC method comprising of acetonitrile, dichloromethane (40:60, v/v) and 0.1% phosphoric acid as the mobile phase. Standard charnatin was eluted at retention time 13.117 min using the mobile phase. The HPLC chromatogram of GMT exhibited peaks at 1.196, 2.058, 2.169, 3.108, 3.519, 5.591 and 13.121 min which occur due to the presence and elution of

several phytoconstituents that could be eluted out using the mobile phase. The peak at 13.121 min was found due to the presence of charantin in GMT. The quantitation of the charnatin was done from the calibration curve of peak area obtained from standard charantin and it was found that GMT contained 8.6 mg charantin per 500 mg of GMT (1.72 %). This suggests a sufficient amount of charantin in the formulation.

This concentration of charnatin might be mainly instrumental for the antidiabetic action of GMT in experimental models. Though the other herbs present may contribute towards the action of the formulation. The presence of Jamun (*Eugenia jambolana)* also contributes to reasonable amount of antidiabetic activity in products.

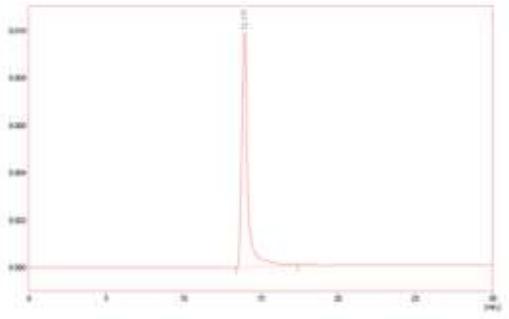

Figure 1 HPLC chromatogram of charantin

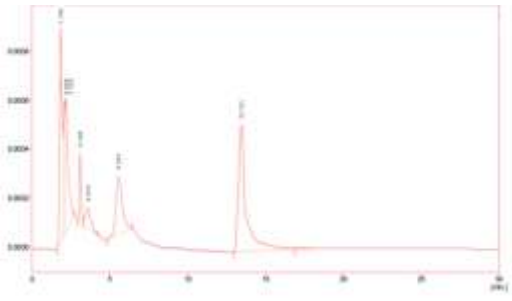

Figure 2 HPLC chromatogram of GMT

Evaluation of antidiabetic action of GMT

Table 3 shows the effect of GMT and standard drug on glucose tolerance as compared to the normal saline control at different hours in alloxan induced experimental diabetes model in rats.

Table 3 Effect of GMT on OGTT

Groups	Treatment / dose	Blood glucose (mg/dl)			
		0 h	0.5 h	1.0 h	2 h
I	Normal control	88 ± 1.3	122 ± 1.2 (↑40.0 %)	110 ± 1.4 (↑30.0 %)	90 ± 0.9 (↑3.33 %)
II	Diabetic control	177.2± 0.8	206.9 ± 1.6 (↑16.57 %)	220 ± 1.02 (↑23.88 %)	184.1 ± 0.8 (↑3.85 %)
III	Glibenclamide, 10 mg/kg	232 ± 1.9	293 ± 2.26 (↑26.06 %)	289 ± 1.5 (↑ 24.35%)	285.4 ± 2.6 (↑ 22.64%)
IV	GMTEE 200 mg/kg	164.3± 0.9	210.6 ± 1.09 (↑27.84 %)	237.9 ± 0.9 (↑44.25 %)	194.1 ± 0.78 (↑17.91 %)

Values are average ± SD of 6 readings

A glucose tolerance test measures the level of glucose in your blood when you are fasting and then 2 hours after drinking a liquid containing a specific amount of glucose. During digestion, the carbohydrates that you eat are broken down into glucose (and other nutrients).

The results of antidiabetic activity of GMT by alloxan induced diabetic model are shown in table 4.

Table 4 Effect of GMT on blood glucose

	Level of blood glucose (mg/dl)				
Groups	**Initial**	**Day 1**	**Day 5**	**Day 10**	**Day 15**
Control	68.76 ± 6.02	63.03±9 .31	64.68± 9.83	65.00 ±7.39	63.46 ±5.86
Diabetic control	247.74 ±8.83	260.26± 14.73	283.83 ±4.76	307.18 ±8.07	311.26 ±4.71
Glibencl amide	246.02 ±3.87	247.63 ± 7.83	219.22 ±5.39	187.8 ± 8.20	176.12 ±9.28
GMTE E	248.83 ±8.38	250.47 ± 5.55	237.21 ±8.40	202.36 ±5.82	190.01 ±5.78

Values are average ± SD; n =6

Alloxan is considered to be the most common chemical substance to induce diabetes in experimental animals. It has been proven that alloxan can lead to rapid depletion or degeneration of the β cells of the islets of Langerhans thereby causing diabetes (Szkudelski, 2001). The level of blood glucose was found to decrease significantly in the diabetic rats when compared to control at the end of the 15th day of study. GMTEE was able to decrease blood sugar by 23.62% while the standard drug glibenclamide could reduce it by 28.12%. This makes it evident that the polyherbal formulation GMT was almost equipotent to the standard drug.

Alloxan-induced diabetes is a form of insulin-dependent diabetes mellitus that occurs as a result of alloxan administration or injection to animals. Alloxan causes diabetes by a mechanism which basically involves partial degradation of the beta (β) cells of pancreatic islets and subsequent compromise in the quality and quantity of insulin produced by these cells.

The drug might therefore be acting by preventing the degeneration of the β pancreatic cells thereby an effective treatment of diabetes mellitus.

Conclusion

From the present investigation various standardization parameters such as physicochemical standards like total ash, acid insoluble ash, water & alcohol soluble extractive values, phytochemical analysis, and pharmacological evaluation were carried out, it can be concluded that the formulation Glucomap tablets contains good characteristics and it may be harmless for human use.

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References

Anonymous. Quality Control Methods for Medicinal Plant Materials. World Health Organisation. Geneva, (1998) 25-28

Chandana M. Puttaraju, Manorama Eti. WHO 75 gram OGTT-A single step procedure for screening and diagnosis of gestational diabetes mellitus. Int J Reproduction Contraception Obstetrics and Gynecology. 2015, 4(6). DOI: http://dx.doi.org/10.18203/2320-1770.ijrcog20151307

https://maharishiayurvedaindia.com/products/glucomap; assessed on 13/01/2021

Mehta S, Singh RP, Saklani P. Phytochemical screening and TLC profiling of various extracts of Reinwardtia indica. Int J Pharmacogn Phytochem Res, 2017; 9(4): 523-527.

Patel S, Patel T, Parmar K, Bhatt Y, Patel Y, Patel NM. Isolation, characterization and antimicrobial activity of charantin from Momordica charantia Linn. fruit, Int J Drug Dev Res, 2010; 2(3): 629-634.

Szkudelski, T., 2001. The mechanism of alloxan and streptozotocin action in B-cells of the rat pancreas. Physiol. Res. 50, 536.

Zold EL, Horvath A, Bacsadi B, Csupor D. Development and validation of a HPLC-UV method for quantification of charantin in momordica charantia products. 2019; DOI: 10.14232/tnpr.2019.af