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Development and Evaluation of Tablets Containing Powders of *Benincasa Hispida* and *Murraya Koenigii*: A Novel Anti-Diabetic Product

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***Abstract*: Diabetes mellitus is a group of metabolic disorders of fat, carbohydrate and protein that results from defects in insulin secretion, insulin action or both. Diabetes mellitus is reported with a reduced life expectancy, morbidity, health complication and overall drop in quality of life. The International Diabetes Federation predicted a huge rise in diabetic population in coming years. Treatment of Diabetes involves diet control, exercise, use of insulin and oral hypoglycemic drugs. The existing treatment options are reported with ineffectiveness against long term Diabetes mellitus complications, side effects and rise in cost. Studies made on plant parts reported with enormous possibilities of treating Diabetic mellitus with formulations containing natural ingredients. The effectiveness, lack of side effects and low cost is making plant based anti-diabetic products popular across the world. The study attempts to develop oral anti-diabetic tablets containing plant based materials as active ingredients and major excipients. The developed product may overcome the drawbacks of existing synthetic anti-diabetic agents. Along with safety, better management of Diabetes mellitus may be possible with the proposed product.**

***Keywords*: Diabetes mellitus, cost, plant**

1. Introduction

Diabetes Mellitus is a group of metabolic disorders of fat, carbohydrate and protein characterized by hyperglycaemia, glycosuria, hyperlipidaemia, negative nitrogen balance and ketonaemia resulting from defects in insulin secretion, action or both [1], [2]. It is associated with a reduced life expectancy, morbidity, health complications and overall drop in quality of life [3].

Non-insulin dependent diabetes (protypical complex multifactorial disease) is one of the chronic diseases which encompass individuals who have insulin resistance and absolute deficiency in insulin. According to WHO estimates, India has 41 million people suffering from diabetes death and this will increase to 70 million people by 2025 and expected to double by 2030 [2]. The greatest increase will be expected in India from 19.4 million to 57.2 million [3]. Type 2 diabetes receives injections if their disease cannot be controlled by diet, exercise and oral medication. The oral hypoglycemic drugs include sulphonyl ureas, biguanides, thiazolidinediones and miscellaneous drugs, which are considered to have many side effects like upset of stomach, constipation, skin rash or itching, weight gain, tiredness or dizziness, risk of liver disease [4].

Lifestyle modification is the most cost effective intervention for the prevention of DM in high risk groups. However, control of DM with diet, weight control and physical activity may be sufficient for most of the patients. Population with Type 1 DM requires daily administration of insulin to regulate the amount of glucose in their blood. Patients with Type 2 DM receive injections, if their disease cannot be controlled by diet, exercise and OADs. The OADs are reported with side effects like upset of stomach, constipation, skin rash or itching, weight gain, tiredness or dizziness, risk of liver disease etc. In spite of the tremendous strides in the modern medicines there is no effective remedy by which we can achieve a tight glycaemic control without possible adverse effects. The existing OADs are becoming less effective over time, along with concerns over safety and tolerability. Hence possibilities for alternative options are wide open in case of oral anti-diabetic products.

The studies made on plant parts are reported with enormous possibilities of treating DM effectively and safely. The effectiveness, lack of side effects and low cost is making plant based anti-diabetic products popular across the world. Worldwide, about 800-1200 plants are reported to be used for the treatment of DM whose mechanisms of action were found to be similar to the conventional OADs. The anti-diabetic agents from natural sources have been reported to contain a vast range of compounds such as alkaloids, polyphenols, flavanoids, terpenoids, coumarins, carotenoids and other constituents which show reduction in blood glucose levels. The different mechanism of actions includes inhibition of the renal glucose reabsorption, inhibition of the β-galactosidase and alpha amylase, stimulation of insulin secretion from β-cells of islets, regeneration or repairing the pancreatic β-cells [4] which may increase the size and number of cells in the islet of langerhans.

The *Benincasa hispida* known as ash gourd is nutritionally and medicinally important plant. Being low in calories and with

the possible isolation of chemical constituents like lupeol, beta-sitosterol, phenolic acids, flavanoids, and terpenoids may be useful for diabetes and obese patients. The *Murraya koenigii* leaves consist of isolated carbazole alkaloids like mahanimbine, mahanine, mahanimbicine which may act as anti-diabetic agents [4]. The investigation revealed that the combination of these plants is not fully utilized as a potential anti-diabetic agent in pharmaceutical formulations.

The world is shifting towards much safer and effective options available from the nature. For every synthetic ingredient or excipient added in present day pharmaceutical formulations, there may be possible replacement available from nature. The effective research in the area of herbal formulation with the help of modern pharmaceutical technology may bring out more effective and safe products.

2. Materials and Methods

A. Collection of plant materials

The fruit of *Benincasa hispida* (Ash gourd), the leaves of *Murraya koenigii* (Curry leaf), the seeds of *Artocarpus heterophyllus* (Jackfruit seed), the leaves of *Aloe barbadensis* and the seeds of *Plantago ovata* will be obtained locally and shall be identified and authenticated from Ratheesh Narayanan, Payyanur College, Payyanur.

B. Preparation of Plant Material

The fruit of *Benincasa hispida* was collected manually peeled to separate its seeds, inner pulp and pulp. The pulp dried and further crushed to powder. The fresh leaves were collected and shade dried at normal room temperature. The dried leaves were crushed to powder [4].

C. Formulation of the Tablet

Table 1
Composition for granules with 10 % w/v binder solution

S. No.	Ingredients	Formulation Code		
		DF_1	DF_2	DF_3
1	Benincasa hispida powder	200	200	200
2	Murraya koenigii powder	300	300	300
3	Artocarpus heterophyllus seed starch paste	q.s	-	-
4	Standard starch paste	-	q.s	-
5	Aloe gum	-	-	q.s
6	Starch powder	10 %	10 %	10 %
7	Plantago ovata	5 %	5 %	5 %
8	Talc	2 %	2 %	2 %
9	Lactose	2 %	2 %	2 %
10	Magnesium stearate	2 %	2 %	2 %

The composition of formulation and formulation codes were summarized in the Table 1, 2 & 3. The wet granulation technique was selected to prepare the granules. The ingredients were weighed as per the requirement. The powders of *Benincasa hispida***,** *Murraya koenigii* and starch powder (5 %) were placed in a clean dry mortar and milled them by hand. Added quantity sufficient of binder solution and mixed it with finger until the mixture was converted to a coherent mass. The coherent mass was screened using sieve no#12 to get the wet granules and later dried in a hot air oven at 60 ± 2 ºC. The dry granules were obtained and weighed. Granules and fines were separated by passing through sieve no#16 and sieve no#44 and added a quantity of the fine equivalent to 15 % weight of granule retained on sieve no#44 to the granules and mixed well. The remaining 5 % of starch powder, *Plantago ovata* powder, talc, lactose and magnesium stearate were added to the granules and mixed well. The granules were evaluated for granule size determination, bulk density, tapped density, angle of repose and hausner ratio. A GMP certified ten station rotary punching machine was used for compressing the granules. The weight and thickness settings were adjusted according to the requirements. The sufficient quantities of granules were taken and mixed well before transferring into the hopper to compress. The compressed tablets were collected and evaluated for physical characterization, weight variation, friability, tablet thickness, tablet hardness and disintegration time [5]-[8].

Table 2
Composition for granules with 12.5 % w/v binder solution

S. No.	Ingredients	Formulation Code		
		DF4	DF5	DF6
1	Benincasa hispida powder	200	200	200
2	Murraya koenigii powder	300 mg	300 mg	300 mg
3	Artocarpus heterophyllus seed starch paste	q.s	-	-
4	Standard starch paste	-	q.s	-
5	Aloe gum	-	-	q.s
6	Starch powder	10 %	10 %	10 %
7	Plantago ovata	7.5 %	7.5 %	7.5 %
8	Talc	2 %	2 %	2 %
9	Lactose	2 %	2 %	2 %
10	Magnesium stearate	2 %	2 %	2 %

Table 3
Composition for granules with 15 % w/v binder solution

S. No.	Ingredients	Formulation Code		
		DF_7	DF_8	DF_9
1	Benincasa hispida powder	200	200	200
2	Murraya koenigii powder	300 mg	300 mg	300 mg
3	Artocarpus heterophyllus seed starch paste	q.s	-	-
4	Standard starch paste	-	q.s	-
5	Aloe gum	-	-	q.s
6	Starch powder	10 %	10 %	10 %
7	Plantago ovata	15 %	15 %	15 %
8	Talc	2 %	2 %	2 %
9	Lactose	2 %	2 %	2 %
10	Magnesium stearate	2 %	2 %	2 %

D. Selection of best tablets formulation

Based on the evaluated parameters, best tablet formulation was selected and subjected for *in-vivo* study.

E. In-vivo study

1) Toxicity studies of the developed tablet

OECD guidelines for acute oral toxicity 423 (Acute toxic class method) was adopted. Female Albino Wistar rats (6) weighing between 150 - 200 g were used for acute toxicity study of the developed tablets. The experinmental animal room conditions were maintained for the animals with temperature: 22 ± 3, relative humidity: 30 % - 70 % and 12-hour light and dark cycle. The dose was prepared 1 ml / 100 g of the body weight. The animal studies were conducted with the Institutional Animal Ethics Committee (IAEC) approval (CPCSEA/IAEC-17/18-12) and all efforts were done to minimize animal suffering.

2) Induction of diabetes

Animals was administered orally with high fat diet (HFD) (fat 20 %, cholesterol 5 %, glucose 5 %, fructose 5 %, glutamine 5 %, and methylthiouracil 1 %) for 10 days. For all assays the physiological saline solution of alloxan is prepared in a concentration of 100 mg/mL. Freshly prepared solution of alloxan (55 mg/kg body weight) was injected into caudal vein and 15 min later, injected (i.p.) to rats with 0.4 U insulin, then 2.5 and 5.0 h later administered orally respectively with 25 % glucose at 10 mL/kg for decreasing mortality rate resulted from hypoglycemia and hyperglycemia in rats with injection of alloxan. The fasting blood glucose was tested and the rats were grouped according to their blood glucose levels. After the injection of Alloxan, the surviving rats with blood glucose concentration more than 200 mg/dl of blood is considered as diabetic rats and selected for further pharmacological studies.

3) Anti-diabetic study Procedure

Animals were divided into 4 groups of 6 rats in each group. Group I received drinking water (normal control) and received normal pellet diet throughout the course till 21 days. Group II act as diabetic control [HFD for 10 days followed by Alloxan monohydrate (55mg/kg dissolved in normal saline) is injected into the caudal vein of overnight fasted rats] received along with the high fat diet started before the study. Group III were served as the standard drug (Acarbose 25mg/kg p.o) treated group in diabetic rats with high fat diet started before the experinment. Group IV received polyherbal tablet p.o as well as Alloxan and high fat diet. Blood sample was collected at weekly intervals from treatment groups by retro-orbital puncture method under anesthesia till the end of study. In the continuous 21 days of drug treatment, blood glucose level of all animals were determined at the 0, 7^{th}, 14^{th}, 21^{st} days by using Dr. Morepen glucometer. The food and water intake was monitored daily for each rat during the experimental period. The in-vivo parameter body weight was measured [9].

F. Biochemical estimation

Blood was collected from treatment groups by retro orbital puncture method, centrifuged to separate serum and supernatant are used for subsequent analysis of blood parameters Viz. plasma glucose, glycated hemoglobin (HbA1c), plasma triglycerides, plasma total cholesterol (TC), high density lipoprotein-cholesterol (HDL-C) by commercially available reagent kits. Low density lipoprotein- cholesterol (LDL-C), very low density lipoprotein- cholesterol (VLDL) was calculated by using Friedwald's formula [9].

G. Statistical analysis

All the results were shown as mean ± standard error of mean (S.E.M). The results were analyzed for statistical significance by one-way analysis of variance (ANOVA) in graph pad prism software version 7. Statistical multiple comparison were performed by using dunnett's tests with respect to normal control, diabetic group and standard group. The P value less than 0.05 ($p<0.05$) were found to be statistically significant and were recorded systematically.

3. Results

The preformulation and the post formulation parameters were analyzed and the best formulation was selected as DF_1. The evaluation studies were performed for the developed granules (DF_1 to DF_9) and tablets (TDF_1 to TDF_9) were compressed. The derived properties of the developed granules from DF_1 to DF_9 had significant differences. It was evident from the investigation, all the developed formulations were having good flow property and can be compressed to tablets. The prepared tablets (TDF_1 to TDF_9) were evaluated and selected quality control parameters i.e. the weight variation, friability, hardness, thickness and disintegration time were within the acceptable limits. The formulation TDF_1 had hardness, thickness, and friability comparable with standard compressible tablets. The disintegration time was less when compared with other formulations. The stability studies also confirmed the stability of formulation TDF_1 at both study conditions. Even elevated temperature did not cause any stability problem for DF_1. Based on these results available from evaluation studies, the formulation TDF_1 may be suggested as the best and recommended for i*n-vivo* study. The parameters for the formulation was tabulated in the table.

Table 4
Table showing the pre formulation parameters of the formulation DF_1

S. No.	Pre formulation parameters	Values
1.	Average granule diameter	10.0 ± 0.12 µm
2.	Bulk density	0.24 ± 0.10 g/ml
3.	Tapped density	0.25 ± 0.11 g/ml
4.	Compressibility index	3.17 ± 0.12
5.	Angle of repose	20.64º± 0.12
6.	Hausner ratio	1.0 ± 0.15

*= All the values are expressed as mean ± SD, n=5

The developed tablets from TDF_1 to TDF_9 were carrying strong aroma which was characteristic of *Murraya koenigii* leaf powder. The colour of the tablets (TDF_1 - TDF_9) was dark green. The colour of *Murraya koenigii* powder was more or less dark green, which may be imparted on to the developed tablets.

The developed tablets were found to be smooth to partially smooth.

Table 5
Table showing the post formulation parameters of the formulation TDF_1

S. No.	Post formulation parameters	Values
1.	Weight variation	0.549 ± 0.95 mg
2.	Hardness	6.6 ± 0.04 Kg/cm^2
3.	Thickness	3.3 ± 0.1 mm
4.	Friability	0.001 %
5.	Disintegration time	6.5 ± 0.13 min

*= All the values are expressed as mean ± SD, n=5

A. In-vivo study

1) Acute toxicity studies

The studies showed that the single dose of the tablet does not produce any toxic symptoms indicating high margin of safety of extract in rodents. After 24 hours, animals were well tolerated. There was no mortality and sign of toxicity after 14 days. From this LD_{50}, $1/10^{th}$ dose was selected to study the anti-diabetic activity as metabolism of drugs in animals is 10 times more than human individuals. So the dose selected for the present study was 200 mg/kg.

2) Body weight

The mean body weight of rats during dietary manipulation were altered (body weight decreased) in alloxan induced diabetic condition. The treatment of the developed tablet at a dose of 200 mg/kg and acarbose, restored the weight significantly when compared to diabetic control. Statistical multiple comparisons were done with respect to normal control and diabetic control. All comparisons given below were found to be significant.

Table 6
Effect of treatment of the developed tablet on the body weight of rats

S. No.	Name & Treatment	Body weight (gm) after treatment on 21st day
1	Normal control (drinking water)	225.00 ± 5.80
2	Diabetic control (Alloxan + HFD)	174.00 ± 3.00 a*
3	Acarbose + (Alloxan +High fat diet)	234.00 ± 3.16 a*b*
4	Developed tablet +HFD (Alloxan +High fat diet)	236.00 ± 5.5 a*b*

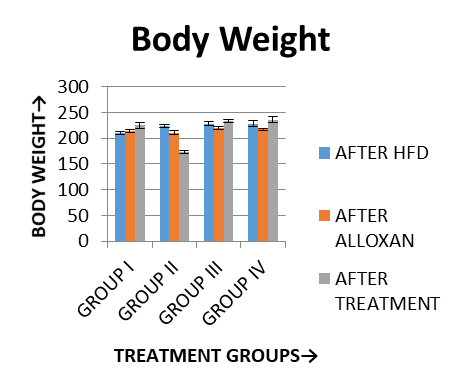


Table 7
Effect of three weeks of treatment of the developed tablet on plasma glucose level (PGL)

S. No.	Name & Treatment	Plasma glucose level (mg/dl) after treatment on 21st day
1	Normal control (drinking water)	75.74 ± 1.90
2	Diabetic control (Alloxan + High fat diet)	390.30 ± 1.22 a*
3	Acarbose + (Alloxan +High fat diet)	162.80 ± 2.60 a*b*
4	Developed tablet + (Alloxan +High fat diet)	165.00 ± 2.40 a*b*

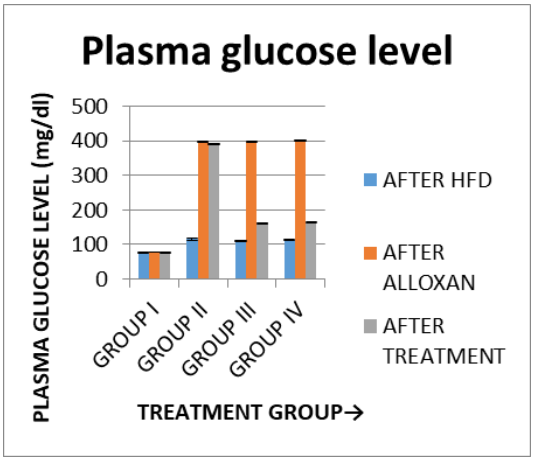


Table 8
Effect of treatment of the developed tablet on plasma cholesterol level

S. No.	Name & Treatment	Plasma cholesterol level (mg/dl) on 21st day
1	Normal control (drinking water)	93.83 ± 1.35
2	Diabetic control (Alloxan + High fat diet)	175.32 ± 2.15 a*
3	Acarbose + (Alloxan +High fat diet)	118.00 ± 2.32 a*b*
4	Developed tablet + (Alloxan +High fat diet)	120 ± 2.50 a*b*

[Values are expressed as Mean ± SEM from 6 rats in each group]

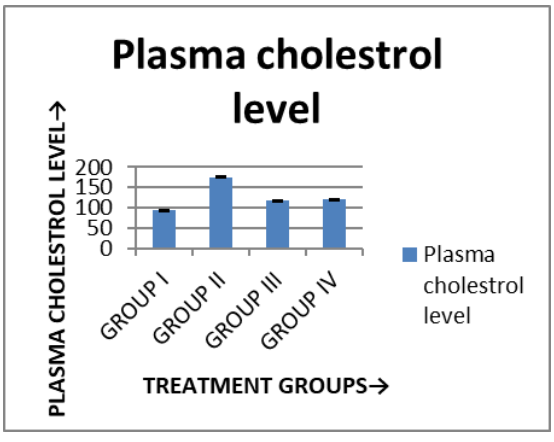
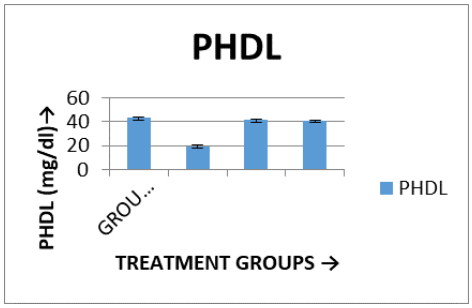


Table 9
Effect of treatment of the developed tablet on PHDL

S. No.	Name & treatment	PHDL after 21 days (mg/dL)
1	Normal control (drinking water)	43.38±2.10
2	Diabetic control (Alloxan + High fat diet)	19.25±1.60 a*
3	Acarbose + (Alloxan +High fat diet)	41.35±1.85 a*b*
4	Developed tablet + (Alloxan +High fat diet)	40.38±1.28 a*b*

[Values are expressed as Mean ± SEM from 6 rats in each group]



4. Conclusion

The literature reviews supported the claimed anti-diabetic activity of selected plant parts which was substantiated by the presence of relevant active constituents through phytochemical screening. The literature reviews also supported the claimed binding and super disintegrant properties of selected natural excipients.

The investigation suggested the possibility of developing effective, safer and economical oral anti-diabetic tablets which may be an alternate for existing synthetic products. Further clinical investigations may be required to expand this proposed oral anti-diabetic formulation to ensure its safety and efficacy in larger population.

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