Research Article

Formulation and Evaluation of Choornam for Antidiabetic Activity

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ABSTRACT

Diabetes mellitus often simply referred to as diabetes—is a condition in which a person has a high blood sugar (glucose) level as a result of the body either not producing enough insulin, or because body cells do not properly respond to the insulin that is produced. In our diabetic research we made an attempt preparing the herbal formulation choornam from selected medicinal plants like pomegranate and watermelon seeds which is having already medicinal properties like preventing prostate cancer, obesity, diabetes, Improves the body's metabolism, cardiovascular system, sexual health and weight loss were used for this studies to understand the synergistic effect of both seed on diabetes. The aim of the study was to formulate and evaluate the choornam for antidiabetic activity.

Keywords: Choornam, antidiabetic, pomegranate, watermelon, formulation.

INTRODUCTION

Diabetes mellitus often simply referred to as diabetes—is a condition in which a person has a high blood sugar (glucose) level as a result of the body either not producing enough insulin, or because body cells do not properly respond to the insulin that is produced. Clinically this disease is associated with a number of chronic complications including nephropathy, neuropathy, retinopathy and cardiovascular diseases. The individual with diabetes has a 25-fold increase in the risk of blindness, a 20-fold increase in the risk of renal failure, a 20-fold increase in the risk of amputation as a result of gangrene and a 2 to 6 fold increased risk of coronary heart disease and ischemic brain damage[1,2,3].

Since time immemorial man has used various parts of seeds and plants in the treatment and prevention of many ailments. Today a substantial number of drugs are developed from seeds and plants which are active against number of diseases. In the developed countries 25 percent of the medical drugs are based on plants and their derivatives and the use of medicinal plants is well known among the indigenous people in rural areas of many developing countries. Although some of the therapeutic properties attributed to seeds and plants have proven to be erroneous, medicinal plant therapy is based on the empirical findings of hundreds and thousands of years. While searching for ant diabetic agent from natural product, encouraging results were obtained from poly herbal formulation with Pomegranate seed and watermelon seed. Pomegranate fruits and seeds are used for conditions of the heart and blood vessels, including high blood pressure, congestive heart failure (CHF), heart attack, "hardening of the arteries" (atherosclerosis), and high cholesterol. It also has anti-diabetic activity and used to prevent prostate cancer, obesity, and weight loss. Water melon seeds will improve the body's metabolism, cardiovascular system and sexual health. It is also used to treat diabetes and sharpening your memory[4,5,6]. In our diabetic research we made an attempt preparing the herbal formulation choornam from selected medicinal plants like pomegranate and watermelon seeds which is having already medicinal properties like preventing prostate cancer, obesity, diabetes, Improves the body's metabolism, cardiovascular system, sexual health and weight loss were used for this studies to understand the synergistic effect of both seed on diabetes.

MATERIALS AND METHODS

METHOD

The formulation consists of fine powder (sieve 36 size) of dried seeds of pomegranate and watermelon in appropriate proportions (2:2) and mixed well. Physical parameters viz, pH, colour, odour, solubility and crude fibre content besides heavy metal and limit test[7,8].

Preparation of choornam

The raw materials such as seeds of watermelon and pomegranate were collected separately. Seeds of watermelon were handpicked and shade dried for about one week. The seeds of pomegranate were collected by pressing the seeds in between the cloth and allowing the juice of the seeds to get separated. After separation the seeds were shade dried for about one week. The seeds were blended in a mixer and passed through sieve no. 36. Both the fine powder was mixed in geometrical proportions ratio (2:2).

Evaluation of physical parameters

1) Determination of pH

The pH of 1% solution of formulated choornam was determined using pH meter.

2) Determination of Ash Values

I. Total Ash Value

2gms of choornam was weighed accurately in a previously ignited and tarred silica crucible. The material was then ignited by gradually increasing the heat to 500-600° C until, it appeared white indicating absence of carbon. It is then cooled in a dessicator and total ash in mg per gm of air dried material is calculated.

II. Acid Insoluble Ash Value

To the crucible containing total ash, 25ml of Hcl was added and boiled gently for 5minutes, and then about 5ml of hot water was added and transferred into crucible. The insoluble matter was collected on an ashless filter paper. This was then washed with hot water until filterate is neutral and the filter paper along with the insoluble matter was transferred into crucible and ignited to constant weight. The residue was then allowed to cool and then weighed.

3) Determination of Extractive Values

I. Water Soluble Extractive Value

5gms of choornam was accurately weighed and placed inside a glass stoppered conical flask. It is then macerated with 100ml of chloroform water for 18hours. It was then filtered and about 25ml of filterate was transferred into a china dish and was evaporated to dryness on a waterbath. It was then dried to 105° C for 6hours, cooled and finally weighed.

II. Alcohol Soluble Extractive Values

Ethanol was used as solvent in place of chloroform water and remaining procedure was the same as that of watersoluble extractive value.

4) Determination of Crude Fibre Content

2gms of accurately weighed choornam was placed in a round bottom flask and then 100ml of 0.128 M sulphuric acid was added and refluxed for 1 hour then filtered through ashless filter paper and the residue was washed with water until filterate becomes neutral. The residue was then weighed (a), ignited to ash and finally the weight of ash (b) was determined. The difference between a and b represented the crude fibre content and was calculated on dry weight basis.

5) Determination of Heavy Metal Contamination

Preparation of sample

Preparation of Choornam solution

The choornam solution was prepared by means of diluting 1gm of choornam to 100ml using distilled water. This is used to carry out limit test for iron and lead and also to perform qualitative test for mercury.

I. Limit test for Iron

Preparation of Standard Solution (20 PPM) One volume of 0.1726% w/v solution of ferric ammonium sulphate solution was diluted in 0.05 M sulphuric acid to ten volume using distilled water.

Procedure

Limit test was performed in Nessler's cylinder. 2ml of test and standard solutions were taken in separate cylinders and then 2ml of 20% solution of citric acid and 0.1 ml thioglycollic acid were added. The solution was then mixed and made alkaline with iron free ammonia, diluted to 50ml with distilled water. It was then allowed to stand for 5minutes and colour obtained in sample was compared with that of standard colour. If the colour produced in test is more when compared to that of standard solution then the sample was said to fail the limit test and said to pass the test if vice versa occurs.

II. Limit Test for Lead

Preparation of Standard (20 PPM)

0.4 gm of lead nitrate was dissolved in water containing 2ml of nitric acid and sufficient water to produce 250ml. About 1volume of

above solution was diluted to 10 volume using distilled water.

Procedure

Limit test was performed in Nessler's cylinder. 1ml of standard lead solution and test solution were taken in separate cylinders and were diluted to 25ml using distilled water and then pH was adjusted to value 3-4 by adding dilute acetic acid or dilute ammonia solution and then diluted to 35ml using distilled water. To both the solutions 10ml freshly prepared hydrogen sulphide solution was added, mixed and diluted with water to 50ml. It was then allowed to stand for 5minutes and viewed downwards over white surface. The colour produced in test solution should not be more intense than that of standard solution, if so then the sample is said to pass the limit test for lead.

III. Test for Mercury

To 10 drops of test solution 6M Hcl was added to get a white precipitate. The precipitate was then treated with 6M ammonia solution. If the colour of precipitate changes to grey or black colour then it indicates the presence of mercury.

Powder Evaluation

Angle of Repose

The angle of repose is the angle formed by the horizontal base of the bench surface and the edge of a cone like pile of granules. The funnel was fixed in its place, 4cm above the bench surface. A cone was formed from 10gms of sample and the base was measured. Formula for angle of repose

$$\theta = \tan^{-1} h/r$$

Bulk density & tapped density

10gms of sample was weighed and filled in a measuring cylinder. The cylinder was tapped manually 100 times on a flat table top surface. Formula

Bulk density= weight/bulk volume

Tapped density= weight/tapped volume

Carr's compressibility index

The bulk and tapped densities are used to calculate the CARR'S COMPRESSIBILITY INDEX (CI). Formula

$$CI = \rho tap - \rho bulk / \rho tap \times 100$$

Hausner ratio

Hausner ratio (HR) is used to provide a measure of the flow properties and compressibility. Formula

$$HR = \rho tap / \rho bulk$$

Where ρtap is the tapped density and ρbulk is the bulk density.

Particle size analysis

The choornum was analysed for particle size by SEM.

In vitro anti-diabetic activity (α-amylase assay)

α-amylase was dissolved in phosphate buffer saline (PBS, 0.02 mol/L, pH 6.8) at a concentration of 0.1 mg/mL. Various concentrations of sample solutions (0.25 mL) were mixed with α-amylase solution (0.25 mL) and incubated at 37 °C for 5 min. Then the reaction was initiated by adding 0.5 mL 1.0% (w/v) starch substrate solution to the incubation medium. After incubation at 37 °C for 3 min, the reaction was stopped by adding 0.5 mL DNS reagent (1% Dinitrosalicylic acid, 0.05% Na_2SO_3 and 1% NaOH solution) to the reaction mixture and boiling at 100 °C for 5 min. After cooling to room temperature, the absorbance (Abs) at 540 nm was recorded by a spectrophotometer. The inhibition percentage was calculated by the following equation:

$$\text{Inhibition (\%)} = [(Abs1 - Abs2)/Abs1] \times 100$$

where, Abs1=sample and Abs2 = control.

RESULTS AND DISCUSSION

Table 1: Evaluation of Polyherbal Choornam

S.NO	Physical Parameters	Values
1.	pH	**6.8**
2.	Ash values	
	I. Total ash II. Acid insoluble ash	**0.78 g** **0.14 g**
3.	Extractive value	
	I. Water soluble extractive value **II.** Alcohol soluble extractive value	**0.026 g** **0.039 g**
4.	Crude fibre content	**0.002 g**
	HEAVY METALS	
5.	Iron (Limit test)	**Within the limit**
6.	Lead (Limit test)	**Within the limit**
7.	Mercury (Qualitative analysis)	**Absent**
	POWDER FLOW PARAMETERS	
8.	Bulk density	**0.35**
9.	Tapped density	**0.47**
10.	Hausner ratio	**1.34**
11.	Carr's compressibility index	**25.53**
12.	Angle of repose	**24.13**

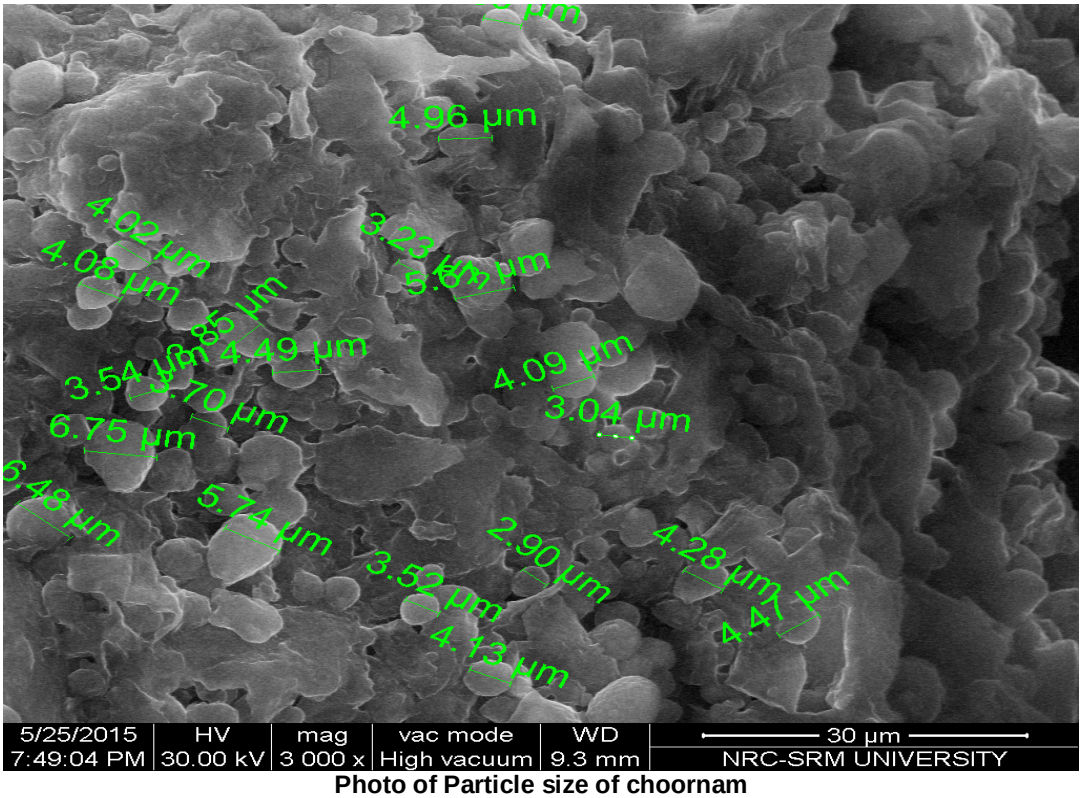


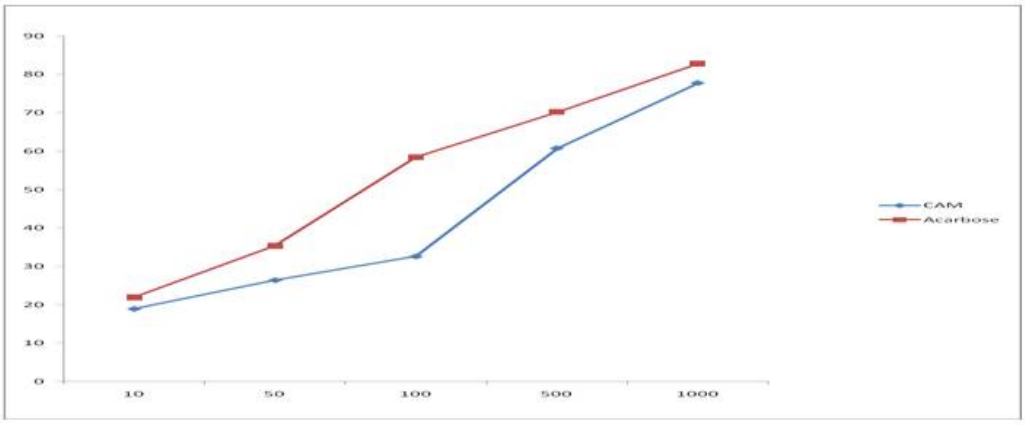
Photo of Particle size of choornam

Table 2: Particle size analysis

S.NO	Sample	Values
1	Choornam	2-7µm

Table 3:

In vitro anti diabetic assay						
Compounds	**Concentration**	**Absorbance**	**% inhibition**		**CAM = Choornam aqueous methanol extract**	
CAM	10µg	0.079	18.98			
	50µg	0.087	26.43		CAM	Acarbose
	100µg	0.095	32.63	10	18.98	21.95
	500µg	0.163	60.74	50	26.43	35.36
	1000µg	0.287	77.71	100	32.63	58.44
	Control	0.064		500	60.74	70.23
				1000	77.71	82.79
ACARBOSE	10µg	0.082	21.95			
	50µg	0.099	35.36			
	100µg	0.154	58.44			
	500µg	0.215	70.23			
	1000µg	0.372	82.79			
	Control	**0.064**				



Graphical analysis of anti diabetic assay

The pH of polyherbal choornam was found to be 6.8. The physicochemical constituent such as ash values, extractive values were found to be 0.78 and 0.039% w/w respectively. The crude fibre content was found to be 0.002. The heavy metal analysis was done which was within the limit and mercury was absent. This shows the product will be safe for medication. The powder flow parameters such as bulk density, tapped density, Hausner ratio, carr's compressibility index and angle of repose was found to be 0.35, 0.47, 1.34,25.53, 24.13 respectively. Particle size analysis of choornam was found to be 2-7µm and analysis was done by FESEM.

CONCLUSION

Present study deals with formulation and evaluation of the choornam from selected medicinal plants like pomegranate and watermelon seeds. Results indicate that the herbal formulation choornam showed moderate anti-diabetic activity by α-amylase assay. The evaluation of choornam showed all the parameters were within the WHO limit. Further broad study and *in-vivo* analysis could establish this plant as commercial sources of anti-diabetic study

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