Research Article CODEN: IJPRNK ISSN: 2277-8713
Divya Jyothi, IJPRBS, 2013; Volume 2(4): 39-47 IJPRBS

INTERNATIONAL JOURNAL OF PHARMACEUTICAL RESEARCH AND BIO-SCIENCE

DESIGN, DEVELOPMENT AND EVALUATION OF ANTIDIABETIC LIQUID ORAL PREPARATION FROM EXTRACT OF FENUGREEK

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Accepted Date: 27/07/2013; Published Date: 27/08/2013

Abstract: Present investigation is aimed at development of suitable liquid oral dosage form from ethanolic extract of fenugreek (*Trigonella foenum-graecum*). Fenugreek seeds were extracted by soxhlation method using ethanol as solvent. Ethanolic extracts were subjected to preliminary phytochemical screening to detect the presence of various active constituents. Extracts were evaluated for invitro- antidiabetic activity by determining alpha-amylase inhibitory activity. Based on the alpha-amylase inhibitory activity, amount of fenugreek extracts were chosen for formulation of liquid orals. Three different liquid oral formulations have been prepared from ethanolic extracts of fenugreek using various vehicles such as glycerin, hydro alcohol and propylene glycol. All the prepared formulations were evaluated for measurement of pH, specific gravity & stability in order to select the best liquid oral of fenugreek. Among three Liquid oral formulations glycerin based liquid oral formulation of fenugreek showed good elegance, more palatability due to higher viscosity with pH in which extracts remains stable. Results of stability study of all the liquid oral formulations indicate good stability without turbidity at storage temperature.

Keywords: Fenugreek, Liquid orals, phytochemical screening, alpha -amylase inhibition

PAPER-QR CODE

Corresponding Author: Ms. DIVYA JYOTHI

Access Online On:

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How to Cite This Article:

Divya Jyothi, IJPRBS, 2013; Volume 2(4): 39-47

INTRODUCTION

A vast variety of pharmaceutical research is directed at developing new dosage forms for oral administration. Liquid oral dosage forms are more effective and are used commonly by young children's or the elderly to over come problem of swallowing the solid oral dosage forms there by increasing the patient compliance[1]. Pant drugs and formulations are frequently considered to be less toxic and free from side effects than synthetic one. Some drugs of plant origin in conventional medical practice are not pure compounds but direct extracts or plant materials that have been suitably prepared and standardized [2].

Fenugreek (*Trigonella foenum-graecum*) has been cultivated and used medicinally and ceremonially for thousands of years in Asian and Mediterranean cultures. Its leaves and seeds are used to treat diabetes in Ayurvedic and other traditional medical systems. Based on the WHO recommendations hypoglycemic agents of plant origin used in traditional medicines are important [3]. Fenugreek seed powders are widely prescribed by ayurvedic physicians for the treatment of diabetis. There are no single constituents in the fenugreek extract to which antidiabetic activity can be attributed. Different constituents such as alkaloids, saponins, 4-hydroxyisoleucine and coumarins are found to be responsible for antidiabetic effect of fenugreek. This study provides new approach to the development of formulations containing the crude plant extract which can give cheaper medicines containing most of the antidiabetic constituents than the isolated constituents. Hence present study is aimed at developing the oral liquid dosage form from extracts of fenugreek seeds which has better palatability, patient compliance and inexpensive than other dosage form from fenugreek.

MATERIAL AND METHODS

Collection and authentification of plant material

Seeds of *Trigonella foenum- graecum* were collected from local market and botanical identity was confirmed by Dr. Nagalaxmi, Botany Department St. Agnes College, Mangalore.

Preparation of extract

Fenugreek seeds were coarsely powdered and then subjected to exhaustive soxhlation using ethanol as solvent for several cycles. After completion of the extraction, it was filtered and concentrated using rotary vacuum evaporator.

Preliminary Phytochemical screening (Qualitative Analysis) [4]

The alcoholic extracts of the fenugreek seeds were subjected to preliminary phytochemical screening for the detection of various plant constituents as per standard procedure [4] and their results are given in Table 1.

Evaluation of α-amylase inhibitory activity

Ethanolic extract of fenugreek was subjected to α-amylase inhibitory assay by CNP-G3 [2-chloro-4-nitrophenol α -D-maltotrioside] method [5]. This assay is based on the principle that pancreatic α-amylase hydrolyses the 2-chloro-4-nitrophenol α -D-maltotrioside (CNP-G3) to release 2-chloro-4-nitrophenol and form 2-chloro-4-nitrophenol α -D-maltoside (CNPG2), maltotriose and glucose. The rate of formation of the 2-chloro-4-nitrophenol is measured at an absorbance of 405 nm using a microplate reader.

Test sample preparation: A sample stock solution of 1218µg/ml was prepared. Briefly, 6.09mg of plant extract (sample) was weighed, dissolved and volume was then made up to 5ml with distilled water.

Pre-incubation mixture was prepared which contained distilled water/positive control/test sample of various concentrations and 0.1488 U of α-amylase enzyme in 250 mM phosphate buffer (pH 5.0). The contents of the plate were mixed and pre-incubated at 39°C for 30 minutes. Following pre-incubation, substrate (CNP-G3) was added to a final concentration of 126.96 µM and the plate was incubated at 39°C for 30 minutes. The absorbance was measured at 405 nm in a microplate reader (Versamax, Molecular Devices, USA). A control reaction was carried out without the test sample.

The % inhibition of the enzyme was calculated as follows:

$$\% \text{ inhibition} = \frac{\text{Absorbance (control)} - \text{Absorbance (test)}}{\text{Absorbance (control)}} \times 100$$

The concentration of acarbose and plant extracts required to inhibit 50% of α-amylase activity under the conditions was defined as the IC_{50} value. The α-amylase inhibitory activities of plant extracts and acarbose were calculated and its IC_{50} values were determined by using log-probit analysis. Results are shown in Table 2.

Formulation of liquid oral preparation[6]

Liquid orals of *Trigonella foenum-graecum* seed extract were formulated using different solvents (vehicles) such as mixture of ethanol and water (1:3), propylene glycol, glycerin which are named accordingly as LO1, LO2, LO3 and formula for preparing the liquid oral is given in Table 3. Based on the invitro alpha amylase activity, amount of extract to be used for preparation of liquid orals were chosen so that each 5ml of the preparation contains 500mg of the extract. In all liquid oral formulations methy paraben, propyl paraben are used as preservatives, aspartame is used as sweetening agent, Disodium EDTA is used as sequestering agent, peppermint oil is used as flavouring agent and sunset yellow as colouring agent. Solubility of the preparations was checked by observing the clarity of solution visually. All the prepared liquid oral preparations were then subjected to evaluation of production quality as per official standards.

Evaluation of oral herbal liquid formulations containing fenugreek seed extract:

Three liquid oral formulations of fenugreek LO1, LO2 and LO3 prepared using ethanol and water (1:3), propylene glycol, glycerin respectively were subjected to evaluation for various parameters such as physical appearance (colour, odour and taste), pH, specific gravity and viscosity[7-9].

Determination of pH

The pH of herbal oral liquids was measured using a digital pH meter. The pH method was calibrated using distilled water, buffer (at pH 4 and 9) pH till constant reading.

Determination of viscosity

Ostwald viscometer was used to determine the viscosity of all samples of oral liquid. The method was followed as per the standard procedure

Determination of specific gravity

Pcynometer was used to determine the specific gravity at 25ºC. It was determined dividing the weight of sample (expressed in gm) by the weight of water (in ml).

Results of evaluation of all three liquid oral formulations are given in Table 4.

Stability testing of oral herbal liquid dosage form

Stability study of prepared liquid orals was carried out for 3 days. The liquid oral preparations were kept at different temperature and relative humidity for short term stability study at 4ºC, 47ºC and at room temperature. Humidity was kept at 75% RH. The parameters checked were turbidity, colour and taste. Liquid oral preparations were stored in ambered colour glass bottle.

RESULTS AND DISCUSSION

Phytochemical analysis of fenugreek seed extracts

Ehanolic extracts of fenugreek were subjected to qualitative tests for the identification of various phytochemical constituents as per standard procedure. The result indicated the presence of presence of alkaloids, phenols, flavonoids, tannins, steroids, carbohydrates, proteins and amino acids in fenugreek extract as shown in Table 1.

Table 1: Results of Qualitative Tests for Phytoconstituents

Sr. No	Tests	Inference
1.	**Alkaloids**	
	a) Dragendorff's test	+
	b) Hager's test	+
	c) Wagner's test	+
	d) Mayer's test	+
2.	**Carbohydrates**	
	a) Benedict's test	+
	b) Fehling's test	+
	c) Molisch's test	+
3.	**Proteins and Amino acids**	
	(a) Biuret test	+
	(b) Million's test	+
4.	**Flavanoids**	
	a) Shinoda test	+
5.	Saponins	+
6.	**Steroids**	
	a) Liebermann -Burchard's test	+
	b) Salkowski reaction	+
7.	**Tannins**	+
8.	**Starch**	+

9.	**Phenolic compounds**	
	a) Ferric chloride test	+
	b) Lead Acetate test	+
	c) Gelatin test	+

Evaluation of α-amylase inhibitory activity

Ethanolic extracts of *Trigonella foenum - graecum* obtained from the soxhlation were subjected to evaluation of α-amylase inhibitory activity in vitro by CNPG3 method. Five different concentration were tested, the extract showed good inhibitory effect at all the tested concentrations (50, 100,250, 500 and 1000 µg/ml), at a higher concentration of 1000 µg/ml the maximum inhibitory effect was observed which showed significant α-amylase inhibitory activity (65.83%) with IC_{50} value of 252.91µg/ml as shown in Table 2.

Table 2: α-amylase inhibition assay of fenugreek extract and acarbose

Sample code	Concentration (µg/ml)	% Inhibition (Mean±SEM)	IC_{50}(µg/ml) (95%confidence Interval)
Acarbose (Positive control)	0.1	34.88±4.79	0.18 (0.14-0.22)
	0.25	55.83±3.16	
	0.5	74.64±2.15	
	1	90.36±0.62	
Ethanolic extract of *Trigonella foenum graecum*	50	23.45±2.35	252.91 (187.21-346.64)
	100	36.43±3.43	
	250	55.83±2.46	
	500	61.79±0.74	
	1000	65.83±4.13	

Note: SEM- standard error of mean; IC_{50}-half maximal inhibitory concentration.

Formulation and evaluation of Liquid oral preparations

Three liquid oral formulations (LO1, LO2, LO3) from fenugreek extract were prepared using mixture of ethanol and water, propylene glycol and glycerin as solvents using different ingredients and evaluated for measurement of pH, Viscosity, specific gravity & stability as shown in Table 3 and 4 respectively.

Table 3: Liquid oral preparations from *Trigonella foenum – graecum*

Fenugreek liquid oral preparations Each 100 ml contains			
	Formulations		
Ingredients	LO1	LO2	LO3
Ethanolic extract Fenugreek	10g	10g	10g
Methyl paraben	0.25g	0.25g	0.25g
Propyl paraben	0.05g	0.05g	0.05g
Disodium EDTA	0.5g	0.5g	0.5g
Aspartame	0.01g	0.01g	0.01g
Peppermint oil	0.005ml	0.005ml	0.005ml
Sunset yellow	q.s	q.s	q.s
Ethanol	25ml	1ml	1ml
Propylene glycol	25ml	upto100ml	------
Distilled water	upto 100ml	5ml	5ml
Glycerin	------	-----	upto 100ml

Note: LO1: Hydroalcoholic based liquid oral; LO2: Propylene glycol based liquid oral; LO3: Glycerine based liquid oral.

Table 4: Evaluation of liquid oral formulations containing Fenugreek seed extract

Evaluation parameters	Formulations		
	LO1	LO2	LO3
Colour	Yellow	Yellow	Yellow
Odour	Aromatic	Aromatic	Aromatic
Taste	Sweet taste	Sweet taste	Sweet taste
Clarity	Translucent	Transparent	Transparent
pH*	5.51±0.005	5.60±0.01	5.54±0.01
Specific gravity(g/ml)*	1.015±0.01	1.050±0.03	1.206±0.045
Viscosity(cps)*	4.098±1.15	13.319±1.0	97.388±1.0

Note: *Values are mean ± SD, (n=3); LO1: Hydroalcoholic based liquid oral; LO2: Propylene g lycol based liquid oral; LO3: Glycerine based liquid oral.

Three formulations had yellow in colour, pleasant flavor and sweet taste. Among the three liquid oral formulations g lycerine based formulation (LO3) showed more palatability due to higher viscosity (97.388cps) than hydroalcoholic based formulation and propylene glycol (LO2) based formulations .Specific gravity of hydroalcohol, propylene glycol, glycerine based liquid oral formulations were found to be 1.015±0.01, 1.050±0.03, 1.206±0.045 with pH value 5.51±0.005, 5.60±0.01, 5.54±0.01 respectively.

Stability testing of oral herbal liquid dosage form

All the liquid oral preparations were subjected to short term stability study at 4ºC, 47ºC and at room temperature for 3days. Humidity was kept at 75% RH. The results of stability study of final Liquid Oral form of drugs indicate no change in colour, taste of herbal liquid dosage form and no turbidity was found at storage temperature. There was no change in pH, viscosity and specific gravity throught the period of stability testing.

CONCLUSION

Liquid oral formulations prepared from extracts of seeds of fenugreek using different vehicles good elegance, stability and better palatability with glycerine as vehicle. Hence from this study it can be concluded that liquid oral could be one of the suitable dosage form for *Trigonella foenum –graecum* seed extract containing all the antidiabetic constituent in it and preferably prepred using glycerine as vehicle. The development of this type of herbal formulations will mark an important advancement in the area of phytopharmaceuticals.

ACKNOWLEDGMENT

I am thankful to Nitte gulabi shetty memorial institute of pharmaceutical sciences, Deralakatte, Mangalore for

providing all the facilities to carry out the work.

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