LINUM USITATISSIMUM IS THE BEST GLUCOSE LOWERING PLANT AS COMPARED TO *NIGELLA SATIVA, TRIGONELLA FOENUM-GRAECUM* AND THEIR MIXTURE

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Abstract

In vitro anti-diabetic activities of *Nigella sativa* (Black seed) (NS), *Trigonella foenum-graecum* (Fenugreek) (TF), Linum *usitatissimum* (*Linseed*) (LU) and Mixture of NS and TF were studied in their acidic extracts. For this purpose a spectrophotometric method using ortho-toluidine reagent was employed. Calibration curve method was applied to determine the glucose content of all seeds. Results of *in vitro* study revealed that all seeds have their own glucose content. Maximum glucose content was present in mixture of NS and TF while minimum content was of LU. The already present content of glucose, affects the hypoglycemic activity of seeds. The anti-diabetic activity of seeds and their mixture varied with glucose concentration in acidic medium. Although maximum anti-diabetic ability was shown by LU. Mixture of NS and TF showed moderate glucose absorbing ability in acidic medium.

Introduction

World health organization (WHO) has declared that diabetic patients are increasing day by day and it is one of the major causes of death after cancer and heart disease. Pakistan is facing a similar problem and up to 2025, about 15000,000 patients will be in our country. At present no cure for this disease is available and scientists are still unsure for the exact cause of this disease. 49% patients of Type 2 diabetes (Non-insulin dependent diabetes mellitus) is controlled by oral medication, while 40% take insulin injection and 10% by diet and exercise. Present study is based on oral medication in terms of herbal medicines.

Medicinal plants are very important for the treatment of different diseases. Present research work consists of three seeds of herbs *i.e. Nigella sativa* (NS) (Black seeds), *Linum usitatissimum* (LU) (*linseed*s) and *Trigonella Foenum-graecum* (FG) (Fenugreek). These plants have been used from centuries for different diseases and diabetes is one of them. These plants are rich source of organic and inorganic compounds (Anwer and Khan, 2007, Fatima *et al.,* 2004) and these metals, non-metals and organic compounds play a key role for curing different diseases.

Nigella sativa (Black seeds) is considered to be a perfect medicine since it was recommended by the Prophet Mohammad (Peace be upon Him) over 1400 years ago. He in his divine wisdom said 'use this black seed; it has a cure for every disease except death'. *Nigella sativa* contain over 100 valuable nutrients (Sharma, 2009), they strengthen the immune system and have different activities like anti-histamine, anti-bacterial anti-tumor, anti-inflammatory, anti-microbial and anti-diabetic (Akash *et al.,* 2011).

Another seeds that was used in the present study was *Linum usitatissimum* (*linseed*). This plant has been cultivated since at least 5000 BC. Bible contains many references of this plant. These seeds are a rich source of nutrition and show different activities like anti-diabetic, anti-cancer, anti-allergic etc. and also used for arthritis, asthma and water retention *etc*. (Bhathena and Velasquez, 2002).

Trigonella foenum-graecum commonly known as methi, a very important medicinal plant. Clinical studies also confirm the anti-diabetic activity of the plant (Ismail, 2009). *In vitro* research of Vijayakumar (Vijayakumar *et al.,* 2005) on this plant shows that the plant may have some compounds which behaves as a phosphate analog and stimulates protein-tyrosine phospholylation by virtue of its inhibitory action on phosphatase (PTPase) (Vijayakumar *et al.*, 2005).

Some people use mixture of herbs to cure diabetes so in the present research work anti-diabetic activities of these individual herbs are compared with their mixture, in acidic medium. Anti-diabetic activity of plants can be determined by two different methods i.e. ortho-toluidine test (Khan *et al.,* 2005 and Fatima, 2004) and enzymatic method (Arayne *et al.,* 2007). In the present study, we used a cheap and easy spectrophotometric method of ortho-toluidine reagent.

Materials and Methods

Sample collection: Seeds of *Nigella sativa* (NS), *Trigonella foenum-graecum* (TF) and *Linum usitatissimum* (LU) were collected from local market and cleaned properly with cotton cloth and stored in tightly capped jar.

All chemicals were of analytical grade purchased from Merck and Sigma. All glassware were properly cleaned and rinsed with distilled deionized water before use. Distilled water was subsequently passed through a column of cation exchanger (Amberlite resin IRA-401 from B.D.H. Chemicals) in order to make it free of cations.

Glucose contents of all the seeds were determined by Vis-7220 spectrophotometer. Absorbances were measured at λ_{max} (630nm) using cell of 1cm path length.

Sample preparation:

Ortho-Toluidine reagent: 2.375g of sodium borate + 0.375g thio-urea and 20mL doubly distilled ortho-toluidine were taken in 250mL measuring flask and then made up to the mark with glacial acetic acid.

Glucose solutions: In 250mL measuring flask, 0.25g of glucose was dissolved and made up to mark with deionized distilled water.

To prepare different concentrations of glucose (20, 40, 60, 80, and 100 mg/dL) 20, 40, 60, 80, and 100mL from the above stock solution was taken in 100mL volumetric flask and made up with distilled deionized water.

Acidic extracts of samples: 4.00g of each herb was soaked in 50mL of 0.1M HCl for 24h and filtered with Whatman No. 41 in a measuring flask of 100mL, washed 2-3 times with 0.1M HCl and made the volume up to the mark with same HCl. The solution was shacked well prior to use.
For mixture solution 4.0g of NS and 2.0g of TF were mixed and treated as mentioned above.

Blank solution: 4mL of ortho-Toluidine reagent was mixed with 1mL of distilled deionized water and heated for 15 minutes on preheated water bath and after cooling absorbance was recorded.

Preparation of sample solution: 0.05mL of acid extract of herb was accurately measured with graduated pipette, transferred to a test tube and then 4.00mL of ortho-toluidine was added, volume was made up to 5.00mL with deionized distilled water. The solution was heated for 15min. on a pre-heated water bath. Solution turned green and then absorbance was taken at 630nm against blank solution.
Similarly solutions of other herbs and mixture were also measured.

Preparation of calibration standards solution: For the preparation of standard solutions five test tubes were prepared which contain 0.05mL of glucose having 20, 40, 60, 80, and 100mg/dL of glucose respectively. 4.00mL of ortho-toluidine reagent was added in each test tube, and then volume was made up to 5.00mL. Solutions were heated for 15 minutes on a pre-heated water bath in this way solutions turned green. Absorbance was recorded at 630nm.

Preparation of sample solutions containing added glucose: Five test tubes were prepared containing acid extract of herb and added glucose solutions. For this purpose 0.05mL of acid extract of herbal solution was added in each test tube and then glucose solution of 0.05mL having 20, 40, 60, 80 and 100mg/dL concentration were added respectively in the test tubes then allowed to react for 3 to 4h, 4.00mL of ortho-toluidine reagent was added to each test tube and then after heating for 15 min. on preheated water bath the solution became green and absorbances were measured at 630 nm.

Results and Discussion

Herbal treatment is increasing day by day due to their low side effects. The main object of present study was the idea that some indigenous herbs have the tendency to take up glucose which reflects the decrease in glucose level of the body. Most of the people use herbs and some use their mixture. In the present comparative research study we analyzed individual herbs as well as their mixture.

Results show that all herbs have their own glucose content which was determined by calibration curve method (Fig. 1 & 2 and Table 1). All absorbances were recorded at 630nm. Absorbance for Mixture of NS and TF were found to be 0.270, while for NS, TF and LU it was 0.201, 0.176 and 0.148 respectively. Results reveal that Mixture of NS and TF show maximum glucose content while *Linum usitatissimum* show least content of glucose (Fig. 2). After analyzing glucose content of each herb, the anti-diabetic effect of acidic extract on addition of standard glucose of varying concentrations were analyzed. The decreased in absorbances of added glucose were observed which clearly indicate that all acidic extracts have anti-diabetic compounds. From these absorbances the left amount of glucose was determined by using formula (Concentration of glucose left = Abs. of Sample x Conc. of Standard /Abs. of Standard).

Table 1. Calibration curve method using ortho-toluidine reagent

Concentration of glucose (mg/dL)	Absorbance at 630nm
20	0.06
40	0.102
60	0.144
80	0.195
100	0.277

Table 2. *In vitro* analysis of glucose lowering effect by Ortho-Toluidine method in acidic extract of the three species and mixture of NS and TF

ASG (mg/dL)	AGA (mg/dL)	ASA	CGS (mg/dL)	UOG (mg/dL)
		Nigella sativa (NS)		
20	95	0.220	73.33	21.67
40	115	0.254	99.61	15.39
60	135	0.269	112.08	22.92
80	155	0.297	121.71	33.29
100	175	0.321	115.94	59.05
		Trigonella foenum-graecum (TF)		
20	85	0.149	49.67	35.33
40	105	0.175	68.63	36.37
60	125	0.240	100.00	25.00
80	145	0.273	112.00	33.00
100	165	0.322	116.24	48.75
		Linum usitatissimum (LU)		
20	70	0.180	60.00	10.00
40	90	0.203	79.57	10.43
60	110	0.196	81.92	28.08
80	130	0.190	78.07	51.93
100	150	0.184	66.43	83.57
		Mixture of NS and TF (M)		
20	124	0.310	103.33	20.67
40	144	0.284	111.37	32.63
60	164	0.353	147.08	16.92
80	184	0.322	132.10	51.90
100	204	0.375	135.38	68.62

ASG = Added amount of standard glucose, AGA = Amount of glucose present + added glucose, ASA = Absorbance of sample of herb after adding glucose, CGS = Concentration of glucose left = Abs. of Sample x Conc. of Standard /Abs. of Standard, UOG = Uptake of glucose = (Amount of glucose present + added amount of glucose)-glucose left, mg/dL = milligram per deciliter (mg/100mL)

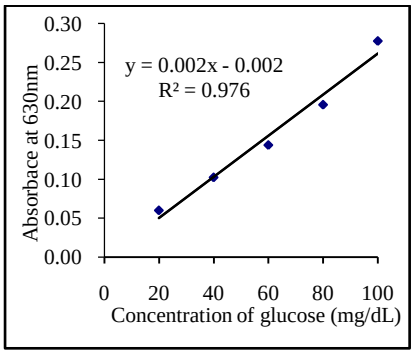

Fig. 1. Calibration curve for glucose

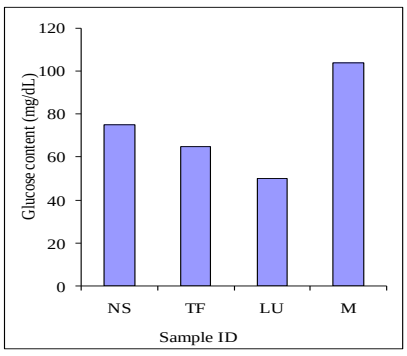

Fig. 2. Glucose content of herbs determined by calibration curve method

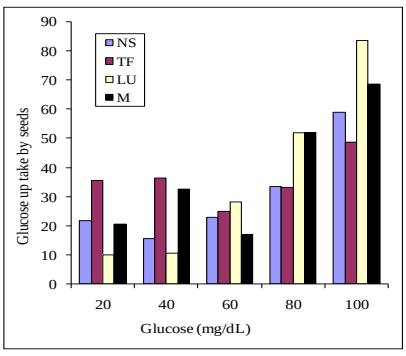

Fig. 3. *In vitro* anti-diabetic activity of NS, TF, LU and M using ortho-toluidine method in acidic extract

While the anti-diabetic activity was determined by using formula (Uptake of glucose = (Amount of glucose present + added amount of glucose)-glucose left) (Khan *et al*., 2005 and Arayne *et al.*, 2007).

Results of *in vitro* study revealed that *Linum usitatissimum* shows significant decrease in glucose concentration in acidic medium. As the concentration of added glucose increases glucose absorbing ability also increases. On addition of 100mg/dL of glucose, *Linum usitatissimum* can lower up to 80 mg/dL of it, while *Nigella sativa* and *Trigonella foenum-graecum* just lowered 60 and 48mg/dL respectively. Mixture of these herbs shows medium reduction of glucose against addition of 100mg/dL up to 68mg/dL (Table 2 and Fig. 3).

In acidic medium all herbs show varying degree of glucose lowering property. *Linum usitatissimum* can lower 10-84% of glucose content. Mixture of NS and TF have ability to lower glucose content 21-69% and individually these herbs show 35-49% and 22-59% anti-diabetic activity respectively (Table 2 and Fig. 3). So the present research shows that *Linum usitatissimum* can be a good anti-diabetic plant.

Conclusions

The spectroscopic method for the assay of glucose was done in the present research work. A simple and quick ortho-toluidine method was employed. *Nigella sativa*, *Trigonella foenum-graecum* and *Linum usitatissimum* all seeds were individually studied for their glucose lowering ability as well as the mixture of NS and TF. Results of the present study clearly indicate that acidic extract of all seeds and their mixture are anti-diabetic. *Linum usitatissimum* show best hypoglycemic activity as compare to *Nigella sativa* and *Trigonella foenum-graecum* but their mixture is better than those of individual herbs. The good hypoglycemic effect of *Linum usitatissimum* may be due to less content of its own sugar.

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