



PRELIMINARY PHYTOCHEMICAL INVESTIGATION AND *IN-VITRO* ANTIDIABETIC ACTIVITY OF AN AYURVEDIC FORMULATION

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Objective: To carry out the preliminary phytochemical analysis and to perform the *in-vitro* anti diabetic activity for the ayurvedic poly herbal formulation.

Material and Methods: Preliminary phytochemical analysis was performed as per the standard procedures. *In-vitro* anti-diabetic activity was done by two methods namely alpha amylase inhibitory assay and alpha glucosidase inhibitory assay by taking acarbose as a standard drug for the ayurvedic formulation. Statistical analysis is carried out by means of graphpad prism software version 6.

Results and conclusion: Preliminary phytochemical analysis showed the presence of alkaloids, glycosides, phytosterols, phenols, flavonoids and terpenoids in the formulation. The poly herbal tablet produced 32.2% inhibition of alpha amylase activity at a concentration of 125 µg/ml and 80.8% inhibition of alpha glucosidase activity at a concentration of 62.5 µg/ml. The present study shows that the selected ayurvedic poly herbal tablets has an anti-diabetic activity comparable with that of allopathic drug - acarbose tablet.

INTRODUCTION

Diabetes mellitus, a metabolic diseases, in which the blood sugar will be higher for a prolonged period of time. It can occur because of two reasons; insufficient insulin production by pancreas or under utilization of the insulin produced. If not treated, it leads to severe complications affecting various organs of the body¹⁻³.

Food is converted into glucose by the liver which is then released into the blood stream. Our body gets energy through the glucose. The regulation of glucose level in the body is done by various hormones, and mainly by insulin produced from pancreas. During diabetes, the body won't be able to regulate the concentration of glucose in the blood and resulting in high blood glucose level.

BGR-34 is a poly herbal drug, jointly developed by scientist of National Botanical Research Institute & Central Institute of Medicinal and Aromatic Plants along with Council of Scientific and Industrial Research.

The important constituents are *Berberis aristata*, *Pterocarpus marsupium*, *Gymnema sylvestre*, *Rubia cordifolia*, *Trigonellafoenum-graecu* and *Tinospora cordifolia*

BGR-34 is a natural dipeptidyl peptidase 4 (DPP-4) inhibitor. It regulates blood glucose level and also possesses cardio-protective action. It prevents the development of diabetic complications by acting as a powerful anti-oxidant. CSIR has launched BGR-34 for the treatment of diabetes. As there are number of drugs available in the market, an attempt was made to compare the anti-diabetic activity of the polyherbal drug (BGR-34) with an available allopathic drug in the market.

MATERIALS AND METHODS

All the chemicals used for the research are of analytical grade. The enzymes were obtained from Enzyme Bioscience Pvt Ltd, Gujrat and Acarbose tablet was obtained from a local pharmacy.

Analysis of Phytochemical Constituents

Phytochemicals are the chemicals produced by various parts of the plants. These bioactive constituents of plants are tannins, steroids, phenols, flavonoids, terpenoids, alkaloids, and glycosides. These compounds have various activities such as antimicrobial, anti-diabetic, antibacterial, antioxidant and other therapeutic activity²⁻⁵.

The phytochemical test for alkaloids, phytosterols, saponins, tannins, mucilage, flavonoids, terpenoids, carbohydrates, proteins, aminoacids and phenols were carried out as per the standard procedures reported³⁻⁵.

in-vitro anti-diabetic assay

α -Amylase inhibitory assay

Alpha-amylase enzyme present in the pancreatic juice and saliva, breaks down large starch molecules (insoluble) into small molecules which can be easily absorbed. α -amylase inhibitors act by lowering the levels of postprandial hyperglycemia. The estimation of inhibition of α -amylase was done by determining the amount of reducing sugar liberated under the assay conditions, in terms of maltose equivalent i.e, decrease in units of maltose liberated. Dinitrosalicylic acid (DNS) method with slight modification was used for the estimation of maltose equivalent^{6&7}.

Procedure

Test samples [BGR-34 (125, 250, 500, 1000, 1250 μ g/ml)] and allopathic drug Acarbose (125, 250, 500, 1000, 1250 μ g/ml)] were added to 500 μ l of sodium phosphate (0.02 M) buffer (pH adjusted to 6.9 with 1% NaCl) having 0.5 mg/ml of α -amylase enzyme solution. All the tubes were incubated at 25°C for 10 min. Then to each tube, 500 μ l of 1% starch solution was added at specified intervals. Incubation was continued for another 10 min. The reaction was arrested by the addition of 1 ml of 3, 5-dinitro salicylic acid (colouring reagent). This was followed by incubation of

the tubes in boiling water bath for 5 mins; cooled to room temperature and diluted with distilled water to get a total of 10 ml. The absorbance of the diluted solution was then measured at 540 nm.

% Inhibition =

$$\frac{\text{Absorbance of control} - \text{Absorbance of extract}}{\text{Absorbance of control}}$$

α -Glucosidase inhibitory assay

α -Glucosidase present in the small intestine catalyzes the final stage of digestion of starch and disaccharides which are abundant in human diet. From the terminal, non-reducing 1,4-linked α -D glucose residues, glucose is released by α -glucosidase. Alpha Glucosidase activity in the biological samples can be directly determined with the aid of α -Glucosidase assay kit, without pre-treatment. In this assay, α -glucosidase enzyme hydrolyses p-nitrophenyl- α -D-glucopyranoside into a yellow colored chromogen which absorbs at 505 nm^{8&9}.

Procedure

The α -glucosidase enzyme activity was carried out by incubating 200 μ l of α -glucosidase enzyme solution with 200 μ l of phosphate buffer (pH-6.9) containing varying concentrations of enzyme inhibitor i.e. sample [BGR-34 (1000, 500, 250, 125, 62.5 μ g/ml)] and Allopathic drug [acarbose (1000, 500, 250, 125, 62.5 μ g/ml)] at 37°C for 60 mins in maltase solution. The reaction was ceased by keeping the mixture in boiling water bath for 2 mins and then cooling. To this mixture, 2 ml of glucose reagent was added and the absorbance was measured at 405 nm.

% Inhibition =

$$\frac{\text{Absorbance of control} - \text{Absorbance of extract}}{\text{Absorbance of control}}$$

Statistical analysis

For both the assays, statistical analysis were carried out by means of graphpad prism software ver.6. All the data were expressed as mean \pm SEM triplicates. The mean values were compared using Mann-Whitney U test.

RESULTS AND DISCUSSION

Qualitative analysis reveals the presence of alkaloids, glycosides, phytosterols, phenols, flavonoids and terpenoids in BGR-34 tablets, whereas saponins, mucilage, carbohydrates, proteins, aminoacids and tannins were found to be absent.

In-vitro anti-diabetic assay

α -Amylase inhibitory assay

BGR-34 produced 32.2% inhibition of α -amylase activity at a concentration of 125 $\mu\text{g/ml}$ whereas acarbose exhibited only 19.15% inhibition in the same concentration. When the concentration is increased to 1250 $\mu\text{g/ml}$ BGR-34 produced only 61.63% inhibition whereas acarbose (allopathic drug) exhibited 83.91% inhibition. The results were depicted in tables 1 & 2 and figure 1.

Table 1: Percentage inhibition of alpha amylase activity produced by the allopathic drug.

S. No	Concentration ($\mu\text{g/ml}$)	Percentage inhibition	SD	SEM
1.	125	19.15	2.0	1.20
2.	250	44.37	1.37	0.79
3.	500	54.55	0.84	0.49
4.	1000	82.41	2.89	1.67
5.	1250	83.91	2.63	1.52

Table 2: Percentage inhibition of alpha amylase activity produced by the poly herbal tablet.

S. No	Concentration ($\mu\text{g/ml}$)	Percentage inhibition	SD	SEM
1.	125	32.25	3.43	1.98
2.	250	42.23	1.75	1.01
3.	500	50.80	2.67	1.54
4.	1000	57.44	0.15	0.09
5.	1250	61.63	1.24	0.72

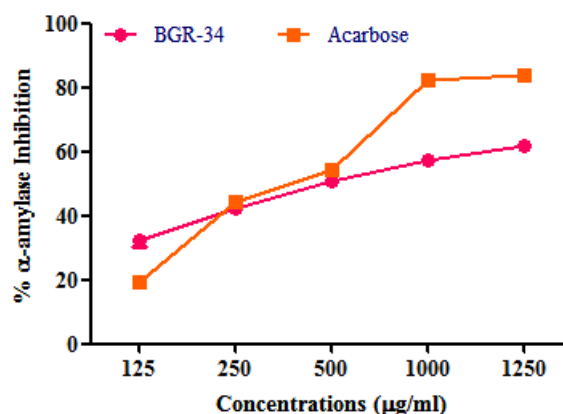


Fig. 1: Comparison of alpha amylase inhibitory activity produced by the allopathic drug and poly herbal tablet.

α -Glucosidase inhibitory assay

From the graph it was observed that the BGR-34 produced a dose dependent increase in the percentage inhibition of the alpha glucosidase enzyme activity and is comparable with that of the allopathic drug, acarbose. BGR-34 has shown 80.8% inhibition of α -glucosidase activity at 62.5 $\mu\text{g/ml}$ and 94.49% at 1000 $\mu\text{g/ml}$, concentrations, respectively. The results were depicted in table 3 & 4 and figure 2.

Table 3: Percentage inhibition of alpha glucosidase activity produced by the allopathic drug.

S. No	Concentration ($\mu\text{g/ml}$)	Percentage inhibition	SD	SEM
1.	62.5	51.55	3.81	2.70
2.	125	63.98	2.97	2.10
3.	250	73.73	3.39	2.40
4.	500	81.36	0.85	0.60
5.	1000	93.22	0.85	0.60

Table 4: Percentage inhibition of alpha glucosidase activity produced by the poly herbal tablet.

S. No	Concentration ($\mu\text{g/ml}$)	Percentage inhibition	SD	SEM
1.	62.5	80.08	4.66	3.29
2.	125	86.44	0.84	0.59
3.	250	87.71	0.42	0.29
4.	500	90.25	1.27	0.89
5.	1000	94.49	2.11	1.49

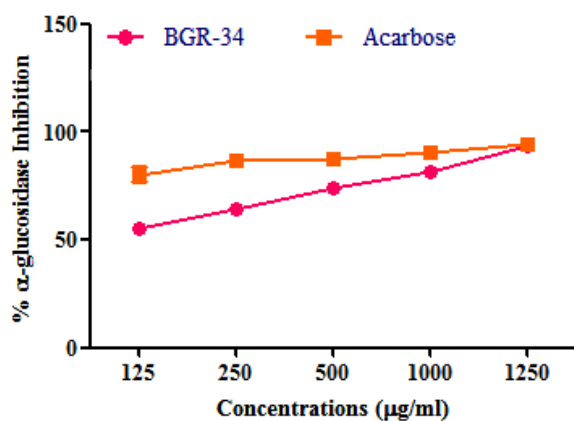


Fig. 2: Comparison of alpha glucosidase inhibitory activity produced by the allopathic drug and poly herbal tablet.

In carbohydrate metabolism the key enzymes present in the small intestine are the pancreatic alpha-glucosidase and alpha-amylase which convert the consumed polysaccharide to monosaccharide. This action by the enzyme causes postprandial blood glucose level elevation due to the absorption of glucose formed in the small intestine. Drugs that have inhibitory activity on both these enzymes possess ability to control the postprandial blood glucose level in type 2 diabetic patients. The available drugs in this category are acarbose and miglitol. Our present study clearly demonstrates that BGR-34 possess pancreatic alpha-glucosidase and alpha-amylase inhibition which confirms that the anti-diabetic action of BGR-34 may act through this pathway by inhibiting the above two enzymes. The safety and efficacy of BGR-34 is further supported by the clinical studies on human beings¹⁰.

Conclusion

The present study shows that the selected ayurvedic polyherbal formulation BGR-34 tablets exhibited anti-diabetic activity comparable to that of Allopathic drug (Acarbose tablet) by α -amylase and α -glucosidase inhibitory assay method. This may be due to the presence of different components in the polyherbal formulation which acts through various pathways.

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نشرة العلوم الصيدلانية جامعة أسيوط



المسح الكيميائي الأولي ودراسة النشاط المضاد لمرض السكري خارج الجسم الحي لتركيبية الايورفيدا

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الهدف: إجراء التحليل الكيميائي الأولي وإجراء النشاط المضاد لمرض السكري خارج لجسم الحي لتركيبية الايورفيدا والمكونة من أعشاب متعددة.

المواد والطرق: تم إجراء التحليل الكيميائي الأولي وفقاً للتجارب القياسية. تم إجراء النشاط المضاد لمرض السكري خارج الجسم الحي بطريقتين هما اختبار مثبطات ألفا أميليز ومقاييسه مثبطات ألفا جلوكوسيداز عن طريق تناول التركيبية والمقارنة مع الأكاربوز كدواء قياسي. تم إجراء التحليل الإحصائي عن طريق إصدار برمجيات الرسم البياني المنشور ٦.

النتائج والاستنتاج: أظهر التحليل الكيميائي النباتي الأولي وجود قلويدات ، جليكوسيدات ، فيتوسترولات ، فينولات ، مركبات فلافونويد وتيربينويد في التركيبية. أدت أقراص الايورفيدا والمكونة من أعشاب متعددة الى ٣٢.٢٪ تثبيط لنشاط ألفا أميليز بتركيز ١٢٥ ميكروغرام/مل و ٨٠.٨٪ تثبيط لنشاط ألفا جلوكوسيداز بتركيز ٦٢.٥ ميكروغرام/مل. توضح الدراسة الحالية أن أقراص الأعشاب المتعددة الأيورفيدا المختارة للدراسة لها نشاط مضاد لمرض السكري يضاهاي نشاط دواء أكاربوز.