Original Article

In-vitro Anti-Diabetic activity of leaves of *Cicer arietinum*

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ABSTRACT

Chickpea, (*Cicer arietinum*), also known as garbanzo bean or Bengal gram, yearly plant of the pea family *Fabaceae*, widely grown for its beneficial seeds. Chickpeas are cultivated in more than 50 countries. Largest areas under chickpeas are India, Pakistan, Ethiopia, Turkey and Mexico. This study incorporates the *Cicer arietinum* leaves anti diabetic activity. It has traditional medicinal uses like anti-diabetic and it is also used in illness to optimize the taste. This work envisaged the anti-diabetic activity of leaves of *Cicer arietinum* by using UV-Visible spectrophotometer at 540nm. The maximum peak of inhibition of alpha-amylase is observed at 10µg/ml.

Key words: *Cicer arietinum*, Alpha amylase, Enzyme inhibition, 3, 5-dinitro salicylic acid.

Diversity in the medicinal plants is the real wealth of India. Since time immemorial many different types of medicinal herbs and their formulation have been used. Therapy using medicinal plants is more esteemed than using synthetic chemicals shown by practical experience and several modern researches works. Medicinal plants growing wild or cultivated are rich in the world, which forms a enormous natural and economical health which must be protected, increased for the development of economy, wealth of nation and health of people [1]. Rich flora of medicinal plants in developing countries is potential source of new biologically active substances [2]. Regarding modern research enterprise, drug development from plants must necessarily suggest a multi-disciplinary approach. As sources of many potent drugs, plants are used medicinally worldwide [3].

Diabetes mellitus is not a single disease but is a group of metabolic disorders affecting a huge number of populations in the world. It is mainly characterized by chronic hyperglycemia, resulting from defects in insulin secretion or insulin action [4]. Even though the cases of diabetes are increasing day by day, except insulin and oral hypoglycemic drugs no other way of treatment has been successfully developed so far. Amylase and glucosidase are mainly used for evaluating the anti-diabetic activity of a particular drug [5].

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MATERIALS AND METHODS

Method of Extraction: 50g of leaf powder has to be subjected to soxhlet extraction process using alcohol as the solvent. Make sure that all the powder should be wet and the solvent passes from powder and settled to the bottom. Due to heating to the apparatus at 55^0C the solvent system again goes at top of powder and by passing powder it again settle at bottom in RBF (Round Bottom Flask) along with the chemical constituents which are dissolve in the solvent system. Heat this apparatus upto the solvent becomes colorless. After that stop heating and keep for 15min for cooling. After cooling, collect all the extract in a clean beaker, then transfer some extract in a petri plate for air dry [6].

***In vitro* anti-diabetic activity:** When blood glucose (sugar) is very high, then it is said to be diabetes (hyperglycemia). The main source of energy in our body is blood glucose, which is obtained from the food that we eat. Insulin is a hormone secreted from beta cells of Islets of Langerhans in pancreas. With the help of insulin blood glucose enters in the cells and utilized as a source of energy [7]. α-amylase and α-glucosidase are carbohydrate hydrolyzing enzymes. α-amylase converts polysaccharides like starch, glycogen to disaccharides by breaking 1, 4-glycosidic linkage by hydrolysis and digest the carbohydrate. α-glucosidase converts this disaccharides to monosaccharides, which are responsible for postprandial hyperglycemia [8]. The agents / drugs that lower the abnormally high glucose (sugar) level in the blood [9].

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α-Amylase inhibitory activity

Chemicals used in the assay: α-amylase, potato starch, sodium acetate trihydrate, glacial acetic acid, sodium hydroxide, 3,5-dinitro salicylic acid, sodium potassium tartarate, ethanol[10,11].

Preparation of reagents

1. **α-amylase solution:** Weigh 27.5mg α-amylase. Take 250ml beaker and pour 100ml distilled water and weighed 27.5mg α-amylase. Stir it for 2 min [10,11].
2. **Sodium acetate buffer:** Weigh 2.72gm sodium acetate trihydrate and add it in 80 ml of distilled water. Adjust the pH at 4.8 with glacial acetic acid. Make up the volume at 100 ml with distilled water [12].
3. **Starch solution (0.1% w/v):** Weigh 0.1gm potato starch and add it in 100ml sodium acetate buffer. Stir to mix properly [10,11,15].
4. **2N NaOH:** Weigh 2gm NaOH and add it in 25ml distilled water and stir it [13].
5. **3, 5-dinitro salicylic acid reagent:** Weigh 1gm 3, 5-dinitro salicylic acid and add it in 50ml distilled water. Weigh 30gm sodium potassium tartarate and add it in above solution by continue stirring. Add 20ml 2N NaOH and dilute this solution to 100ml with distilled water [10,11].

Table 1: Preparation of reagent for *In-vitro* anti-diabetic activity of leaves of *Cicer sarietinum*.

Reagent	Quantity Required
α-amylase solution	0.5ml
Sodium acetate buffer	[Q.S]
Starch solution (0.1% w/v)	0.5ml
2N NaOH	[Q.S.]
3, 5-dinitro salicylic acid reagent	1ml

Preparation of test solution: The stock solution of leaf extract was prepared of 100µg/ml by using ethanol and 70% aqueous ethanol as a solvent. For this, 0.001gm (1mg) semidried drug extract were added in 10ml of solvent. From this stock solution, 5 different concentrations of 2, 4, 6, 8 and 10µg/ml were prepared by pipetting 0.2, 0.4, 0.6, 0.8 and 1ml of stock solution and dilute it up to 10ml [14].

Procedure: In this assay, 0.5ml of test solution of *Cicer arietinum* leaf extract of different concentration such as 2µg/ml, 4µg/ml, 6µg/ml, 8µg/ml and 10µg/ml prepared in ethanol and hydro-alcohol were taken in different test tubes. Then 0.5ml of α-amylase enzyme solution and 0.5ml of starch solution were added and allow standing for 3 minutes for reaction among the drug extract, enzyme and starch. Then add 1ml of 3, 5-dinitro salicylic acid to stop the reaction. At the same time same procedure follow for control, instead of 0.5ml test solution add 0.5ml distilled water. Then measure the absorbance at 540nm using UV- Visible spectrophotometer [14].

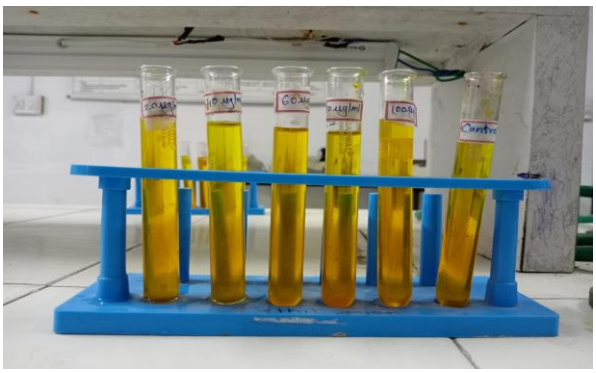


Figure 1: Dilution of Anti-diabetic Activity

Calculation: Inhibition of α-amylase were calculated by using following formula:

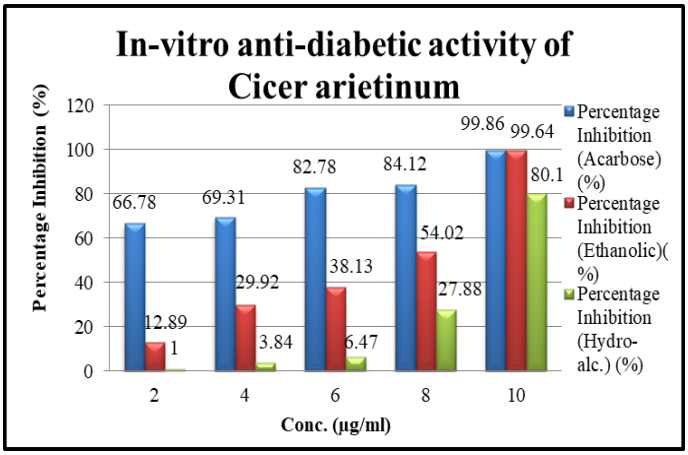
$$\% \text{ Inhibition} = \frac{\text{Abs.of control} - \text{Abs.of test}}{\text{Abs.of control}} \times 10$$

RESULTS

***In-vitro* Anti-diabetic Activity**

Table 2: *In-vitro* anti-diabetic activity of *Cicer arietinum* for ethanolic and hydro-alcoholic extract

Conc. (µg/ml)	Percentage Inhibition (Acarbose) (%)	Percentage Inhibition (Ethanolic) (%)	Percentage Inhibition (Hydro-alc.) (%)
2	66.78	12.89	1
4	69.31	29.92	3.84
6	82.78	38.13	6.47
8	84.12	54.02	27.88
10	99.86	99.64	80.1



Graph 1: *In-vitro* anti-diabetic activity of *Cicer arietinum* for ethanolic and hydro-alcoholic extract.

DISCUSSION

Cicer arietinum plant shows presence of various plant constituents like alkaloids, glycosides, steroids, carbohydrates,

amino acids, inorganic elements. These constituents exhibit anti-diabetic activity [16]. Alpha amylase converts polysaccharide into glucose and maltose. That's why blood glucose level increases and causes the postprandial hyperglycemia. By inhibiting the alpha amylase enzyme we can prevent breakdown of carbohydrate into glucose and maltose [17,18]. Alpha amylase inhibitory assay is firstly applied to study the anti-diabetic activity of the leaves of *Cicer arietinum.* Demonstration of the alpha amylase inhibition by ethanolic and hydro-alcoholic extract was done by this study. This study shows percentage inhibition of alpha amylase. Ethanolic extract shows high percentage inhibition at 10µg/ml i.e. 99.64%. The percentage inhibition increases as concentration increases as shown in table and graph. Hydro-alcoholic extract shows high percentage inhibition at 10µg/ml i.e. 80.10%. The percentage inhibition increases as concentration increases as shown in table and graph [19,20].

CONCLUSION

These studies conclude that, the ethanolic and hydro-alcoholic extract of leaves of *Cicer arietinum* shows anti-diabetic activity by alpha amylase inhibition assay. But the ethanolic extract has high percentage inhibition as compared with hydro-alcoholic extract. Ethanolic extract at 10µg/ml shows high percentage inhibition i.e. 99.64%. The percentage inhibition increases as concentration increases from 2µg/ml to 10µg/ml as shown in table and graph.

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