

Evaluation for Anti-Diabetic Activity in Callus and Suspension Cultures of *Lepidium sativum* L.

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Abstract: The present investigation focused on the ability of callus extract for the production of pharmacologically important phytochemicals and can be used in preliminary screening of anti-hyperglycemic activity of plant species. The callus and suspension culture of *Lepidium* plant was established and was evaluated for anti diabetic potential by testing for inhibitor activity of alpha amylase and glucosidase enzymes. The results indicated that both seedling and callus extracts possessed considerable hyperglycemic activity and can be effective as alternatives in the treatment of diabetes.

Keywords: callus, *Lepidium*, anti-hyperglycemic, alpha amylase inhibitor, glucosidase

1. Introduction

Diabetes mellitus is a serious concern in many countries including India and to control it, traditional methods using medicinal plants are gaining momentum. There are enzyme inhibitors which are used clinically such as Acarbose, Voglibose but because of their known side effects, the search of effective and safer hypoglycemic agents has continued to be an important area of investigations based on the extracts of traditional medicinal plants [1-4]. Since alpha amylase inhibitors inhibit the action of α -amylase enzyme leading to a reduction in starch hydrolysis which shows beneficial effects on glycemic index of diabetic patients. Thus enzymes alpha amylase and α -glucosidase inhibitors are drug design targets in the development of herbal drugs for the treatment of diabetes [5-6].

The literature survey revealed that though several plants showed the anti-diabetic activity but the study involving plant tissue culture based is lacking in this area of research. Studies [7-10] showed that callus extracts of plants possess more potent amylase inhibitor activity. Similarly *in vitro* anti diabetic activity of methanolic extracts of *Cinnamomum zeylanicum*, *Piper betle*, *Artocarpus heterophyllus* and *Artocarpus altilis* was investigated by [11]. Thus finding suitable inhibitors of glucosidase and amylase enzymes with minimum side effects possess a challenge in area of research for a potent therapeutic agent.

Keeping above in view, the present study was undertaken with the following objectives: i) Establishment of callus and suspension culture of *Lepidium* plant ii) Evaluation of alpha amylase and alpha glucosidase inhibition activity.

2. Materials and Methods

2.1 Seed Material

Seeds of the Garden cress (*Lepidium sativum* L.) belonging to Brassicaceae family were collected from the local market. The

seeds were grown under *in vitro* conditions and healthy seedlings of 10 days old were selected for callus induction.

2.2 Callus Culture

The leaf and stem explants were sterilized with 0.1% HgCl₂ for 10 minutes. The seedlings were subjected to 70% alcohol for few seconds. The explants after washing with autoclaved distilled water for three times were used for inoculation. The leaf and stem were cut with sterilized blade and were transferred aseptically to ½ strength of MS media containing IAA+ 2,4 -D + Kinetin as growth regulators (1mg/L). The culture tubes were incubated to 16 hr photoperiod light intensity of 2000-3000 lux and 8 hr Dark period at 25±2^o C, until callus was induced. (Figure 1)

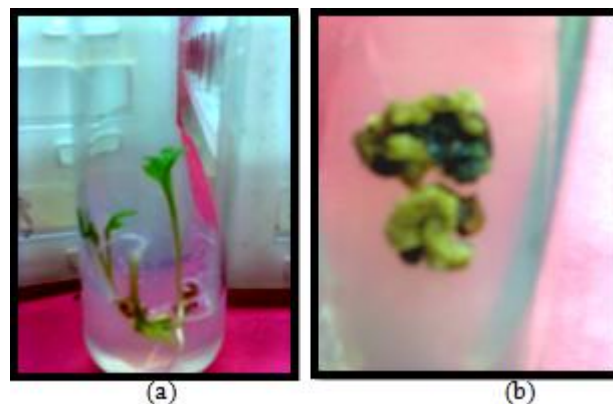


Figure 1: In vitro raised plants from seeds of *Lepidium* : a) Seedling b) Callus culture

2.3 Plant and callus extract

Leaves and callus extract (0.5g each) of *Lepidium* plant was prepared with 80% acetone solvent system and centrifuged at 10,000 rpm for 10 minutes. The filtrate was collected and stored at 4°C till further use.

2.4 Suspension culture

The callus culture was maintained in liquid MS media supplemented with growth regulators IAA+ 2,4 -D + Kinetin (1.0mg/L) on shaker at 25 °C for 15 days. The suspension culture was used to assay for amylase and glucosidase inhibitor activity.

2.5 Amylase inhibitor activity using DNS method [12]

The inhibitor activity was assayed using salivary amylase (1:1 dilution with saline), diastase amylase (1mg/ml), Pancreatic amylase (1mg/ml) using 0.02M Phosphate buffer (pH 6.7). The controls were maintained along with replicates for each set of the experiment and percentage of inhibition was calculated for the same. The reaction mixture consisted of 500µl of plant or callus extract, 500µl of phosphate buffer (pH 6.7) and 500µl of α amylase enzyme (Salivary amylase / Pancreatic amylase / Diastase amylase). The tubes were pre-incubated at 25°C for 15 minutes, 500µl of 1% starch solution was added to each tubes and the reaction was stopped with 1.0 ml of dinitrosalicylic acid colour reagent. The test tubes were then incubated in boiling water bath for 15 minutes and cooled. Absorbance recorded at 540nm. Percentage of amylase inhibition: $(A_{540} \text{ control} - A_{540} \text{ extract}) / A_{540} \text{ control} \times 100$

2.6 Alpha Glucosidase inhibitor assay [13]

Enzyme was extracted from overnight soaked seeds (0.5g) of *Sorghum vulgare* and was crushed using 80% acetone. The sample was centrifuged at 10,000 rpm for 15 minutes and was used as an enzyme source. The enzyme was stored in small aliquots at 4°C. 500µl of plant/callus extract, 500µl of 0.02M PO₄ Buffer, 500µl of glucosidase enzyme (*Sorghum*) were added. The mixture was incubated at 25°C for 10 min. 500µl of starch solution was added and incubated at 25°C for 10 min. Later 500µl of DNSA was added & kept in boiling water bath for 15 min. after that it was cooled. Absorbance was recorded at 540 nm. Percentage of inhibition is calculated as mentioned for amylase inhibition.

3. Results and Discussion

The present study aimed to find potent hypoglycemic agents and to minimize animal sacrifices in the preliminary screening of anti - hyperglycemic activity of plant species. The alpha amylase and glucosidase inhibitor activities were studied using callus and suspension cultures.

3.1 *In vitro* callus cultures

The leaf extracts and *in vitro* grown callus from stem and leaf explants showed inhibitory activities against various amylases used: salivary, pancreatic and diastase. Maximum inhibitory activity for callus extract was recorded with enzyme glucosidase (87.73%) and pancreatic amylase (76.69%) (Figure 2). Similarly plant extracts showed 67.08% inhibition for glucosidase and 61.05% for salivary amylase enzyme. There was also an increase in inhibition activity against pancreatic amylase when compared with plant extracts. Comparable results were obtained in *Costus* callus [9]. These findings clearly indicates that the plant along with their callus culture holds great potential in controlling blood sugar level and strengthens the use of *in vitro* cultures for production of bioactive metabolites which has medicinal applications.

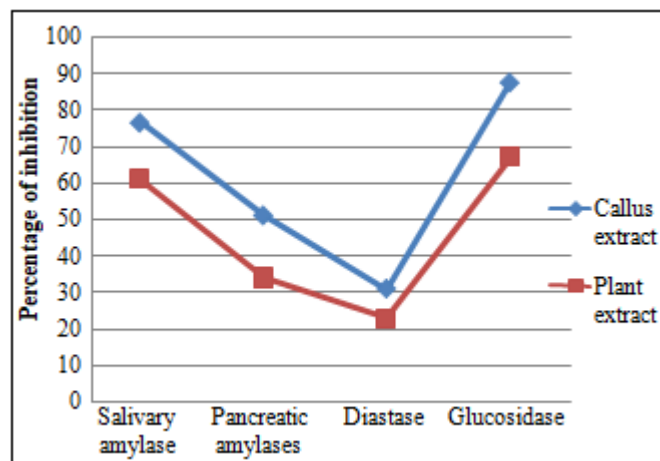


Figure 2: Alpha amylase and glucosidase inhibitory activities in callus and plant extracts

These inhibitory activities detected are found to be very useful in controlling blood sugar level as delay in carbohydrate digestion causes reduction in glucose absorption rate which consequently reduce the postprandial plasma glucose rise [14]. Thus screening for such enzyme inhibitors are useful in controlling diabetes mellitus over many years [15]. The study offers a prospective therapeutic approach for the management of type 2 diabetes mellitus.

3.2 Suspension cultures

Significant results obtained when suspension culture were tested for enzyme inhibition activity using DNSA test (Figure 3). Increase in enzyme activity observed against salivary amylase and glucosidase enzyme with increase in number of days of culture but after 10th day of culture there was decline in the enzyme activity. Minimum activity was obtained with reference to pancreatic (79.54%) and diastase (84.44%) enzyme.

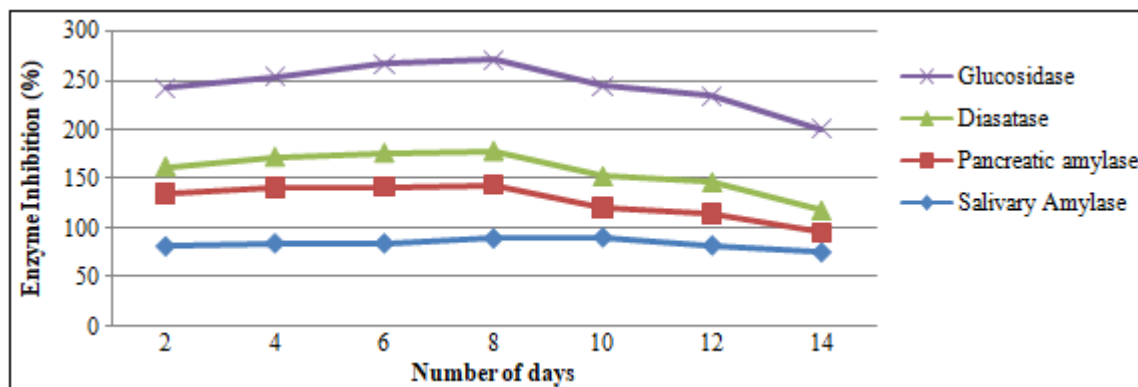


Figure 3: Inhibitory activities of amylases and glucosidase enzymes in suspension cultures

4. Conclusions

Thus the present study indicates significant inhibitory effect for both amylases and glucosidase enzymes under *in vitro* condition supporting the use of *Lepidium* plant as dietary supplement for treatment of diabetes.

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