



**A STUDY ON INVITRO ANTI-DIABETIC ACTIVITY OF CRUDE ACETONE EXTRACT OF RHIZOPHUS SPECIES BY ALPHA AMYLASE INHIBITORY ASSAY.**

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**ABSTRACT**

Diabetes mellitus is a heterogeneous group of diseases characterized by chronic hyperglycaemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action, or both. Alpha amylase inhibitors play major role in the management of postprandial hyperglycemia. The aim of the current study was to study on invitro anti-diabetic activity of acetone extract of rhizopus species by alpha amylase inhibitory assay. Rhizopus sps was cultivated on Sabourds agar medium and inoculated in to Sabourds broth and subjected to fermentation at room temperature for one week. The thick mycelium was separated by filtration and extracted with Acetone solvent to obtain crude extract. The cytotoxicity of crude extract was determined using INS- 1 cells by MTT assay and were cultured in Rose Well Park Memorial Institute medium. The non cytotoxic concentration of the crude extract was used for antidiabetic activity by alpha amylase inhibition assay. The percentage of alpha-amylase inhibition at different concentration of crude acetone extract of Rhizopus species was found to be (200µg/ml) 24.29%. (250µg/ml) 35.08, %. (300µg/ml) 36.34, %. (350µg/ml) 38.84%, (400µg/ml) 41.84, %. (450µg/ml) 43.84. %.

**KEYWORDS:** Diabetes, SDA, DNS, Rhizopus, MTT, RPMI, DMSO, INS-1 Alpha amylase.

**INTRODUCTION**

Diabetes mellitus is a heterogeneous group of diseases characterized by chronic hyperglycaemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action, or both. The effects of diabetes mellitus include long-term damage, dysfunction and failure of various organs. Diabetes mellitus may present with characteristic systems such as thirst, polyuria, blurring of vision, and weight loss. In its most severe forms, ketoacidosis or a non-ketotic hyperosmolar state may develop and lead to stupor, coma and in absence of effective treatment, death. Type 1 diabetes mellitus is characterized by beta-cell destruction caused by an autoimmune process, usually leading to absolute insulin deficiency (the expert competence). This form of diabetes, which accounts for only 5-10% of all diabetes, is a juvenile-onset diabetes; it results from a cellular-mediated autoimmune destruction of the beta-cells of the pancreas by CD4 and CD8 T and macrophages infiltrating the islets.

Gestational diabetes mellitus is defined as any degree of glucose intolerance with onset or first recognition during pregnancy. It is a common condition affecting about 7% of all pregnancies; its detection is important because of associated maternal and fetal complications.

Type 2 diabetes is a complex heterogeneous group of metabolic condition characterized by elevated levels of serum glucose. Alpha amylase is a well-known endoamylase. It is found in a wide variety of microorganisms, belonging to the archaea as well as the bacteria.

**(I) FUNCTIONS OF ALPHA AMYLASE**

Alpha amylase inhibitors play major role in the management of postprandial hyperglycemia. Alpha-amylase is a key enzyme in digestive system and catalyses the initial step in hydrolysis of starch to maltose and finally to glucose. Hence, retardation of starch digestion by inhibition of enzyme such as  $\alpha$ -amylase would play a key role in the control of diabetes. Inhibitors currently in clinical use for example, acarbose, miglitol, and voglibose are known to inhibit a wide range of glycosidases such as alpha-glycosidase and alpha-amylase.

**(II) INSULIN**

Insulin is one of the most potent antidiabetic hormone; its major function is to counteract the concerted action of a number of hyperglycemia-generating hormones and to maintain low blood glucose levels. Insulin is synthesized as a prohormone in the beta-cells of the islets of

Langerhans in response to increased circulating levels of glucose. Then, it is directly infused via the portal vein to the liver, where it exerts profound metabolic effects. These effects are the response of the activation of the insulin receptor which belongs to the class of cell surface receptors that exhibit intrinsic tyrosine kinase activity

### (III) RHIZOPUS

*Rhizopus* is a genus of common saprophytic fungi on plants and specialized parasite on animals. They are found on a wide variety of organic substrates, including "mature fruits and vegetables jellies, syrups, leather, bread, peanuts, and tobacco.

### (IV) 3T3-L1 CELL MORPHOLOGY

3T3-L1 is a cell line derived from (mouse) 3T3 cells that is used in biological research on adipose tissue. 3T3-L1 cells have a fibroblast-like morphology, but, under appropriate conditions, the cells differentiate into an adipocyte-like phenotype.



3t3- l1 cell line

### (V) INVITRO CULTIVATION OF 3T3L1 CELL LINE

The cultivation of 3t3 l1 on the base medium of ATCC-formulated Dulbecco's Modified Eagle's Medium. To make the complete growth medium, add the following components to the base medium: bovine calf serum to a final concentration of 10%. Remove and discard culture medium. Briefly rinse the cell layer with 0.25% (w/v) Trypsin - 0.53 mM EDTA solution to remove all traces of serum that contains trypsin inhibitor. Add 2.0 to 3.0

mL of Trypsin-EDTA solution to flask and observe cells under an inverted microscope until cell layer is dispersed.

### (VI) MTT assay

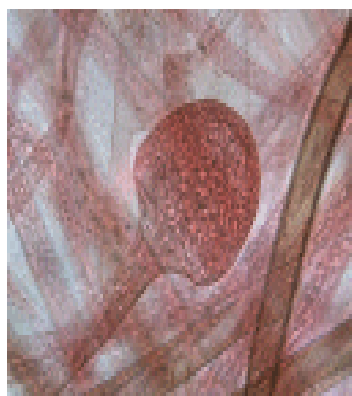
INS- 1 cells were cultured in Rose Well Park Memorial Institute medium, supplemented with 10% fetal bovine serum, penicillin (250 U/ml), Gentamycin (100µg/mL) and amphotericin B were obtained from Sigma Chemicals. All cell culture were maintained at 37°C in a humidified atmosphere of 5% Co<sub>2</sub>. Cells were allowed to grow to confluence over 24 hrs before use. The monolayer cell culture was trypsinized and the cell count was adjusted to 1ml using medium. To each well of 96 well microtitre plates, 0.1ml of diluted cell suspension was added. After 72 hour, the sample solution in wells was flicked off and 50µl of MTT dye was added to each well. The plates were gently shaken and incubated for 4 hours at 37°C in 5% Co<sub>2</sub> incubator. The supernatant was removed, 50 µl of Dimethyl sulphur oxide was added, and the plates were gently shaken to solubilize the formed formazan.<sup>[14]</sup> The absorbance was measured using a micro plate reader at a wavelength of 490- 570 nm. The percentage growth inhibition was calculated using following formula,

$$\% \text{cell inhibition} = 100 - \left\{ \frac{(At - Ab)}{(Ac - Ab)} \right\} \times 100.$$

### (VII) ALPHA- AMYLASE INHIBITION ASSAY

In six test tubes add 200µl, 250µl, 300µl, 350µl, 400µl and 450µl of α amylase solution (1mg/ml phosphate buffer) to this 20µl, 25µl, 30µl, 35µl, 40µl and 45µl of sample was added except blank and mixed well. Pre incubate the prepared mix at 37°C for 20 minutes in water bath. 380µl, 375µl, 370µl, 365µl, 360µl, 355µl of substrate solution (0.5% starch in phosphate buffer) and mix well. Incubate at 37°C for 15 mins. 500µl of DNS (Dinitrosalicylic acid reagent) : (40 mM DNS, sodium potassium tartrate, 0.4% M NaOH) was added in all test tube to stop the reaction and add 500µl of starch and 200µl of Hydrochloric acid in all test tube. Mix well and boil at 100°C for 10mins. Cool the mixture and measure the absorbance in spectrophotometer at 540 nm.

## RESULTS



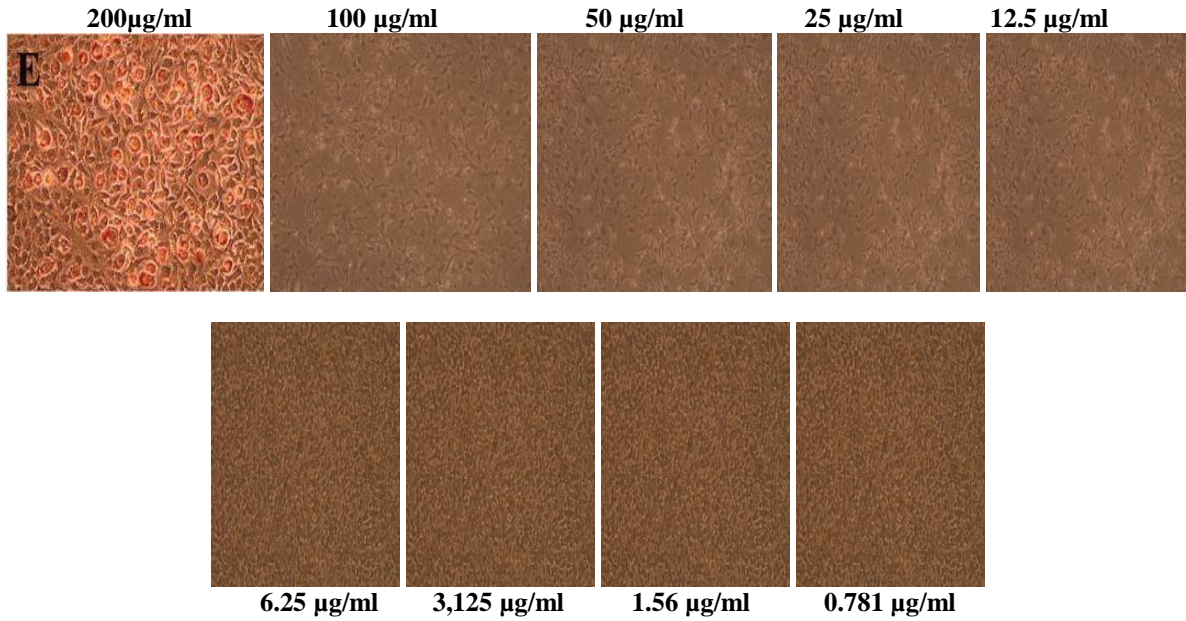
Microscopic



Macroscopic

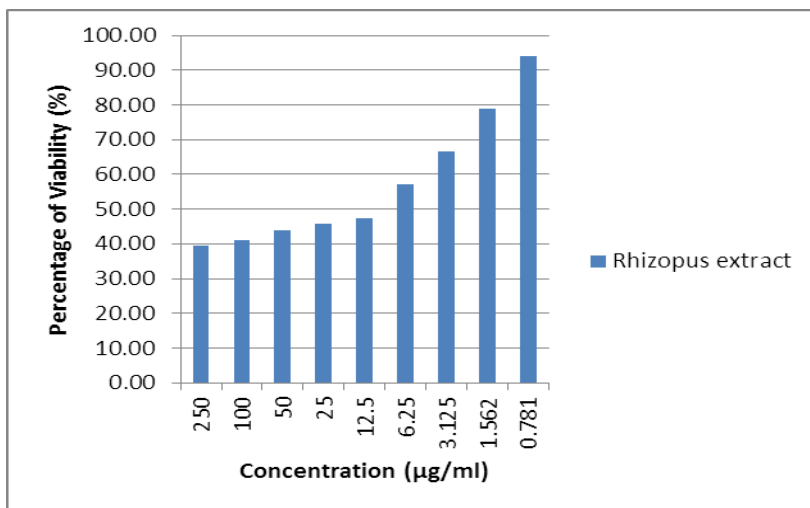


**MTT ASSAY**



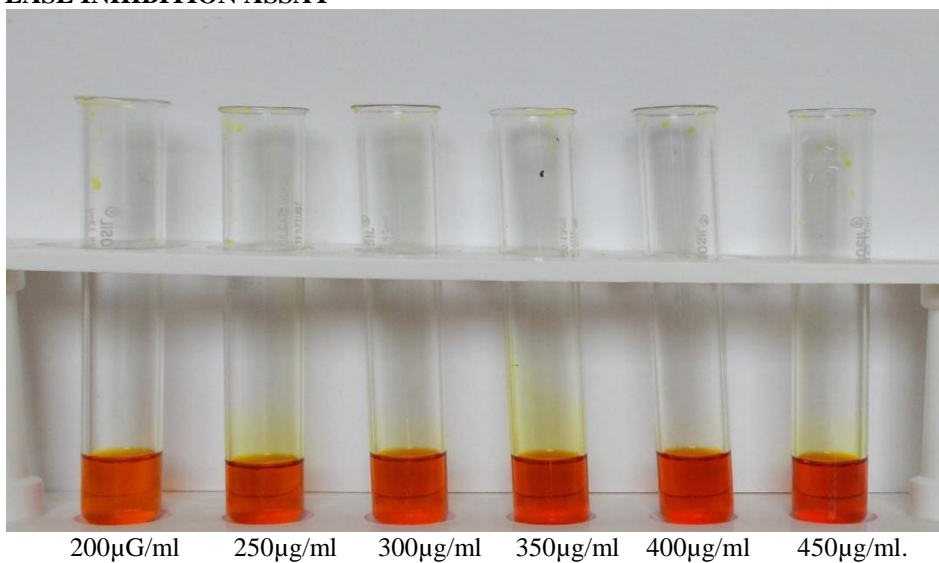
**Table 1: PERCENTAGE OF CELL VIABILITY**

Sample-Rhizopus extract Concentration (µg/ml)	% Live cell Rhizopus extract
250	39.32
100	40.96
50	43.78
25	45.69
12.5	47.51
6.25	57.09
3.125	66.74
1.562	78.79
0.781	94.20



The crude acetone extract of *Rhizopus* exhibited cytotoxicity at different concentrations such as (250µg/ml) 39.32, (100µg/ml) 40.96, (50µg/ml) 43.78, (25µg/ml) 45.69, (12.5µg/ml) 47.51, (6.25µg/ml) 57.09, (3.125µg/ml) 66.74, (1.562µg/ml) 78.79, (0.781µg/ml) 94.20.

**ALPHA-AMYLASE INHIBITION ASSAY**



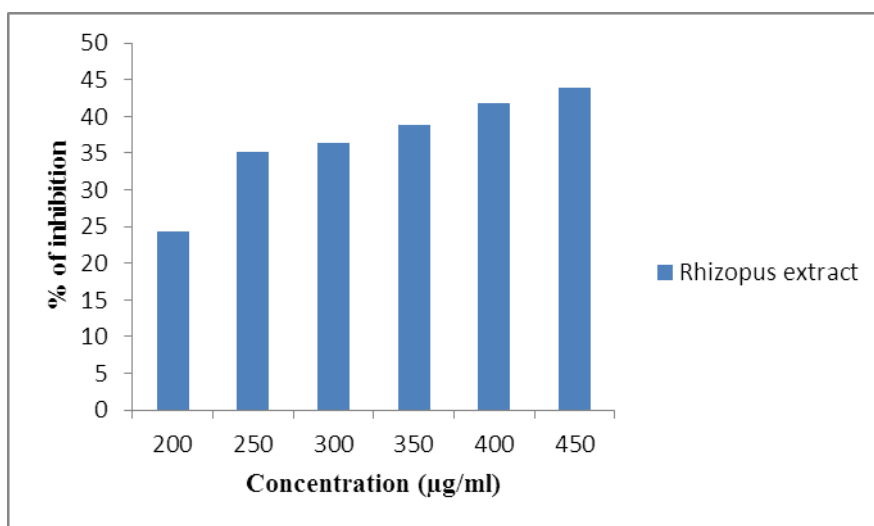
200µG/ml 250µg/ml 300µg/ml 350µg/ml 400µg/ml 450µg/ml.  
**Figure-1: Alpha Amylase Inhibition Assay.**

**TABLE-2.**

S.NO	Concentration(µg/ml)	OD @ 620 nm	Percentage of Inhibition
1	200	0.391	24.29
2	250	0.456	35.08
3	300	0.465	36.34
4	350	0.484	38.84
5	400	0.509	41.84
6	450	0.527	43.83

TABLE-3.

ALPHA AMYLASE ACTIVITY	
Concentration( $\mu\text{g/ml}$ )	% of inhibition Rhizopus extract
200	24.29
250	35.08
300	36.34
350	38.84
400	41.84
450	43.83



The percentage of alpha-amylase inhibition at different concentration of crude acetone extract of *Rhizopus* species was found to be (200 $\mu\text{g/ml}$ ) 24.29%. (250 $\mu\text{g/ml}$ ) 35.08, %. (300 $\mu\text{g/ml}$ ) 36.34, %. (350 $\mu\text{g/ml}$ ) 38.84%, (400 $\mu\text{g/ml}$ ) 41.84, %. (450 $\mu\text{g/ml}$ ) 43.84. %.

### CONCLUSION

The current study was to evaluate the alpha-amylase inhibition of crude acetone extract of *Rhizopus* species at different concentrations. MTT assay reported that the crude acetone extract of *Rhizopus* was found to exhibit 39.32% and 94.20% of cell viability to INS1 cell line at concentration of 250 $\mu\text{g/ml}$  and 0.781 $\mu\text{g/ml}$  respectively. The crude extract showed highest percentage of alpha-amylase inhibition concentration of 450 $\mu\text{g/ml}$ . Based on current study on alpha-amylase inhibition assay using crude acetone extract of *Rhizopus* species it was concluded that crude extract exhibited high alpha-amylase inhibition at concentration of 450 $\mu\text{g/ml}$  with percentage value of 43.83 and the lowest concentration at 200 $\mu\text{g/ml}$  with percentage value of 24.29.

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