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THE EFFECTS OF *MOMORDICA CHARANTIA* CRUDE LEAF EXTRACT ON THE ENZYME KINETICS OF PORCINE ALPHA AMYLASE

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Abstract: Diabetes Mellitus, is classed as a carbohydrate disorder marked by high levels of blood sugar in the body caused by insufficient insulin production (Type 1 diabetes) or insulin resistance (Type 2 diabetes). It is considered as one of the most widespread diseases and numerous researches have been conducted to provide efficient and cost-effective therapeutic remedies and medications for diabetic patients. Treatments by the use of crude leaf extracts of *Momordica charantia* is by far the most broadly investigated and the most commonly acclaimed remedy in Asia. *M. charantia*, also referred to as bitter melon or ampalaya have been found to exhibit hypoglycemic actions. A previous study conducted by DLSU students showed that the crude extracts of *M. charantia* affected the kinetics of the enzyme alpha amylase. Amylases are enzymes responsible for the breakdown of starch to glucose. Alpha-amylase or 1,4-D-glucan glucanohydrolase hydrolyses linear polymers at internal bonds. To further measure its efficiency, the effects of various concentration of crude leaf extract of *Momordica charantia* on the enzyme kinetics of porcine alpha-amylase were determined using the Bernfeld Assay. The Michaelis-Menten constant (K_m) and the maximum velocity (V_{max}) of alpha amylase with starch as a substrate and porcine pancreatin as the source of alpha amylase, with and without the leaf extracts were established using the Michaelis-Menten and Lineweaver-Burk plots. Results illustrated uncompetitive inhibition due to the decrease in the values of K_m and V_{max} . Another proposed model is the possibility of several allosteric effectors. The effects of the crude leaf extract of *Momordica charantia* on the activity of alpha amylase may be valuable in the search for new therapeutic remedies for diabetes

Keywords: alpha amylase; K_m ; V_{max} ; inhibitors

1. INTRODUCTION

Diabetes Mellitus is a disorder marked by high levels of blood sugar in the body due to insufficient insulin production (Type 1 diabetes) or insulin resistance (Type 2 diabetes). It is one of the most widespread diseases and has interested many researchers to pursue studies on finding new cost effective therapeutic remedies. The ingestion of *Momordica charantia* as food ingredient, or in capsule form, or as tea, is by far the most commonly acclaimed alternative remedy for hyperglycemia. *M. charantia*, also referred to as bitter melon or ampalaya has been found to exhibit hypoglycemic actions. Extracts of *M. charantia*, have been found to demonstrate hypoglycemic activity which may offer an alternative treatment for diabetes. Determining the possible targets of *M. charantia* in regulating blood glucose levels is valuable in the search of therapeutic remedies of diabetes as well as enhancing or mediating drugs for better treatments of



such disease. To measure its efficiency, the effects of crude leaf extract of *Momordica charantia* on the enzyme kinetics of porcine alpha amylase were identified. Amylases are enzymes responsible for the breakdown of starch to glucose. Alpha-amylase catalyzes the hydrolysis of internal alpha-1,4-glucan links in polysaccharides yielding a mixture of maltose and glucose. There are two types of amylase in the body: salivary alpha amylase in the mouth and pancreatic alpha amylase in the small intestine. Inhibition of this enzyme therefore will prevent the absorption of glucose from the GI track into the bloodstream. The purpose of the study is to verify the efficiency of crude leaf extracts of *Momordica charantia* as an alpha amylase inhibitor. Specifically the study aimed to determine the type of inhibition exhibited by crude leaf extract of *Momordica charantia* as well as the effects of crude leaf extract of *Momordica charantia* on the K_m and V_{max} of porcine pancreatin alpha-amylase.

2. METHODOLOGY

Sample Collection and Preparation

The plant samples were obtained from a local market in Manila. Thus, growth conditions, farming practices, and specific primary source were all unavailable. The leaves were washed thoroughly and were dried by using tissue paper. The leaves were weighed to approximately 5.00-g and were placed in a beaker with 100-mL distilled water. The solution was boiled for 5-minutes. The solution was then filtered into a clean beaker, and was set aside to cool to room temperature.

Enzyme Assay and Determination of K_m and V_{max}

The Bernfeld Assay was used to determine the effect of the extracts on the K_m and V_{max} of alpha amylase. Starch was used as the substrate and porcine pancreatin was used as the source of alpha amylase. The K_m and V_{max} of alpha amylase were determined using the Michaelis-Menten plot and the Lineweaver-Burke plot. For the Michaelis-Menten, a hyperbolic curve showing the effect of substrate concentration in mg/mL (x-axis) on the rate of reaction in mg/min-mL (y-axis) was constructed. K_m was located by finding the substrate concentration at 50% of the maximum velocity (V_{max}). For the Lineweaver-Burke, the reciprocal of the rate of reaction (y-axis) versus the reciprocal of the substrate concentration was plotted. V_{max} was determined by getting the reciprocal of the y-intercept while K_m is calculated from the negative reciprocal of the x-intercept.

3.0 RESULTS AND DISCUSSION

The K_m and V_{max} obtained from the Michaelis Menten plot at various concentrations of extracts of *M. charantia* are shown in Table 1. Results show a decrease on both the K_m and V_{max} with increasing volume of leaf extract. The decrease in K_m may imply the presence of a constituent that results in increase affinity of starch

for the alpha amylase. The binding of this entity has caused the rate of reaction to decrease as demonstrated by the lowering in the V_{max} . Alternatively, the lowering in the V_{max} may also be attributed to another allosteric inhibitor.

Trial 1				
	0.0mL	0.50mL	1.00mL	1.50mL
K_m (mg/mL)	0.600	0.170	0.370	0.320
V_{max} (mg/mL-min)	0.058	0.034	0.046	0.046
Trial 2				
	0.0mL	0.50mL	1.00mL	1.50mL
K_m (mg/mL)	0.300	0.270	0.130	0.220
V_{max} (mg/mL-min)	0.043	0.017	0.024	0.032

Table 1 Summary of V_{max} and K_m Values from Michaelis Menten equation .

The K_m and V_{max} values obtained from the Lineweaver-Burk Plots are significantly different from that of the Michaelis-Menten Plots in both trials. (Table 2) However; it has still exhibited the same pattern in which it has both decreased K_m and V_{max} values.

Trial 1				
	0.0mL	0.50mL	1.00mL	1.50mL
K_m (mg/mL)	40.5	5.80	12.7	9.17
V_{max} (mg/mL-min)	1.80	0.55	0.80	0.64
Trial 2				
	0.0mL	0.50mL	1.00mL	1.50mL
K_m (mg/mL)	4.47	1.67	0.32	0.68
V_{max} (mg/mL-min)	0.17	0.05	0.04	0.07

Table 4.4 Trial 2: Summary of V_{max} and K_m Values

Both trials have demonstrated a pattern which indicates the presence of an uncompetitive inhibitor which binds specifically to the enzyme-substrate (ES) complex forming an enzyme-substrate-inhibitor (ESI) complex thus reducing its rate of production, explaining the lowering in the V_{max} .

4.0 CONCLUSIONS

The addition of varying amounts of *M. charantia* crude leaf extract decreased the values of the K_m and V_{max} among all sets of trials containing the inhibitor against that of the control. This may imply the possibility of uncompetitive inhibition. In addition, the presence of allosteric inhibitors in the crude leaf extract is also possible. An allosteric effector is capable of changing the shape of the active site of an enzyme, yet making the binding of substrates still possible. These validate the results wherein due to the reduced rate of production, the



value of V_{\max} decreased. The use of known compounds isolated from *M. charantia* as possible is recommended for possible future follow up on this research.

5.0 REFERENCES

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