

Original Research Article

In vitro inhibitory effects of protein isolates from unripe plantain (*Musa paradisiaca*) on key enzymes linked to type-2 diabetes and obesity

ABSTRACT

Aims: This study was carried out to examine and characterize the effects of protein isolates from unripe plantain on enzymes linked with type-2 diabetes (α -amylase) and obesity (Pancreatic Lipase) using an in vitro model.

Place and Duration of Study: Department of Biochemistry, Lagos State University, Ojo, Lagos, Nigeria between July 2019 and January 2020.

Methodology: Proteins were isolated from dried raw flour of unripe plantain, and enzyme inhibition assays were conducted in the presence of protein isolates using α -amylase from *Saccharomyces cerevisiae* and porcine pancreatic lipase. Acarbose and Orlistat were used as the standard inhibitors respectively. Percentage inhibition of the two enzymes and the IC_{50} values were determined. The effects of pH, temperature and salts on the inhibitory activity of the protein isolates were also determined.

Results: The protein isolates showed maximum percentage inhibition on α -amylase activity at 87% (IC_{50} value 0.46 ± 1.9 mg/mL) compared with acarbose with a maximum inhibitory potential of 86% (IC_{50} value 0.51 ± 3.6 mg/mL), and maximum percentage inhibition on pancreatic Lipase at 91% (IC_{50} value 0.73 ± 3.2 mg/mL) compared with Orlistat with a maximum inhibitory potential of 97% (IC_{50} value 0.61 ± 9.4 mg/mL). The optimum pH for both α -amylase and pancreatic lipase inhibitory activity was observed to be pH 9.0, and, the optimum temperature for α -amylase and pancreatic lipase inhibitory activity was observed to be at 40°C. At certain concentrations the inhibitory activities of the unripe plantain protein isolates were affected by the salts.

Conclusion: These results showed the presence of α -amylase and pancreatic lipase inhibitors in unripe plantain which indicates that this plant can be beneficial in the construction of anti-diabetes and anti-obesity medications.

Keywords: Diabetes; Obesity; α -amylase; pancreatic lipase; orlistat; acarbose; unripe plantain; protein isolate

1. INTRODUCTION

Worldwide obesity has more than doubled since 1980. In 2014, over 600 million adults and 42 million children aged 5 years and below were obese [1]. Obesity, particularly visceral adiposity, is associated with insulin resistance. In the world, up to 90% of the patients with type 2 diabetes are either overweight or obese [2]. The incidence of diabetes and obesity worldwide has dramatically increased due to the modern lifestyle and to the increase in the consumption of food that is rich in fat and carbohydrates [3]. One important approach for reducing obesity is to reduce dietary fat or carbohydrate digestion and absorption [4]. Pancreatic lipase is accountable for the breakdown of 50–70% of total dietary fats [5].

Orlistat, a hydrogenated derivative of lipstatin, derived from *Streptomyces toxytricini*, is the only pancreatic lipase inhibitor currently approved for long-term treatment of obesity. Its use can result in up to 10% weight loss when used in combination with a diet and physical activity. The most commonly reported adverse effects of orlistat are a range of gastrointestinal side effects, including steatorrhea, bloating, oily spotting, faecal urgency, and faecal incontinence, as well as hepatic adverse effects (cholelithiasis, cholestatic hepatitis, and subacute liver failure) [6].

Digestion of dietary starch occurs rapidly and leads to postprandial spikes in blood glucose. In diabetes, there is a much developed and prolonged increase in blood glucose levels during the postprandial phase. One of the therapeutic approaches used to lower blood glucose concentration is to decrease the postprandial rise in the blood glucose by inhibiting key enzymes hydrolyzing the dietary carbohydrates [7, 8].

Acarbose is produced by *Actinoplanes sp.* Fermentation [9]. It is widely recognized as a potent inhibitor of several carbohydrate hydrolyzing enzymes. Acarbose interrupts the production of monosaccharides especially glucose, by inhibiting the α -amylase which can help to improve insulin resistance and glycemic index control in diabetic patients [10]. During treatment with acarbose, a high occurrence of troublesome gastrointestinal symptoms such as flatulence, abdominal distension, borborygmus and diarrhea were observed [11].

Natural remedies for diabetes and obesity from plants are gaining popularity as they are effective, cheap and safe when compared to synthetic drugs [12].

Musa paradisiaca is known as Plantain (English), Ogede (Yoruba), Ogadejioko (Igbo), Ayaba (Hausa). It is a perennial tropical plant that is native to India. The fruit of unripe plantain is used in folklore medicine for treating diarrhea, dysentery, intestinal lesions in ulcerative colitis, diabetes, uremia, nephritis, gout, hypertension and cardiac disease [13]. In Nigeria, it is very common to find a diabetic consuming unripe plantain meal to reduce postprandial glucose levels. This is because the propensity of individuals to develop diabetes and obesity is due to the increased consumption of carbohydrate-rich foods with a high glycaemic index [14]. Plantain fruits are good sources of plant phytochemicals, [13] which promote health and well-being. The consumption of the plantain and its product is now on the increase even though it could be eaten singly. Nigerians have adopted various means of including it in their various meals as part of their diets [15].

Despite the traditional usage of unripe plantain in the management of diabetes, there is a dearth of information on its anti-obesity properties, and the consequence of physiological queries on its enzyme inhibitory potentials. This research was therefore set up to study the enzymatic activities of unripe plantain protein isolates in the inhibition of the key enzymes linked to type-2 diabetes and obesity, its mode of action, and the effect of pH, temperature, and salts on its inhibitory activities.

2. MATERIAL AND METHODS

2.1 Plant materials

The unripe plantain (*Musa paradisiaca*) was obtained from the nearby local market at Iyana Iba, Ojo local government, Lagos state and identified and authenticated in the Department of Botany, Lagos State University, Ojo-Lagos, Nigeria.

2.2 Sample preparation

The unripe plantain pulps were sliced and sun-dried for about 4 weeks to a constant weight, and ground with a blender into flour which was termed "raw flour".

2.3 Reagents and Equipment

Dinitrosalicylic acid (DNS), Sodium potassium tartrate, α -amylase, porcine pancreatic Lipase (EC 3.2.1.1), p-nitrophenyl- α -D-glucopyranoside, Folin ciocalteau (Folin-C) reagent, were procured from Sigma Aldrich, Inc. (St. Louis, MO, USA). Hydrochloric acid (HCL), n-hexane, Sodium hydroxide (NaOH), Ethyl alcohol, Sodium chloride, Phosphate buffer, Sodium phosphate, and Soluble starch were gotten from the Reagent storage room, Lagos State University, Ojo. The water used was glass distilled. Equipment used included; Centrifuge (Gulfex medical and scientific, England, Model No 90-1), Blender

(Goodway electrical enterprise ltd, Hong Kong, Model No GJ-1501), Freezer (Haier Thermocool, Nigeria, Model No HTF-379H), pH meter (Eco tester) Electronic compact scale (ATOM A-110C), UV Spectrophotometer (Spectrum LAB S23A, Globe Medical, England), Regulated Water bath (Unigold Medical England, Model No PI-420), and micro millimeter pipette (100µl-1000µl).

2.4 Extraction procedure

2.4.1 Defatting the raw flour

The powdered sample of 50 grams was suspended in 200mL of n-hexane in a beaker covered with an aluminum foil. The suspension was stirred continuously for some minutes and was allowed to settle down overnight. The residue was recovered and excess extract solution was separated by squeezing in a muslin cloth. The defatted unripe plantain sample was spread on a muslin cloth and dried. Total defatting was confirmed by pressing one gram of defatted sample on filter paper and there was no soiling of paper with oil, this indicates complete defatting of the sample.

2.4.2 Preparation of protein isolates

The extract from the defatted powdered plantain fruit was prepared using alkaline extraction and subsequent iso-electric precipitation. Defatted unripe plantain was dispersed in 5 volumes of 1.0M NaCl solution, it was stirred for 15minutes at ambient temperature followed by adjusting to pH 9.5 using 1.0M NaOH, and stirring for 30minutes. The dispersion was centrifuged at 5,000rpm for 1 hour at ambient temperature. The supernatant was filtered through the muslin cloth to remove insoluble material and was adjusted to pH 4.0 with 1.0M HCl to precipitate the extract and centrifuged again at 5,000rpm for 1 hour at ambient temperature. The precipitate was later dispersed in 50% ethyl alcohol (1:5) stirred for 30 minutes and centrifuged at 5,000rpm for 1 hour at ambient temperature. The precipitate was washed several times with distilled water, and adjusted to pH 7.0. Powder forms of the precipitate were stored at room temperature until used for inhibitory assays.

2.5 Alpha amylase inhibition Assay

This assay was carried out using a modified procedure of McCue and Shetty[16]. The starch solution of (0.5% w/v) was derived by stirring 0.25g of soluble starch in 50mL of de-ionized water for 15 minutes. The enzyme solution (0.5unit/mL) was prepared by mixing 0.001g of α -amylase (EC 3.2.1.1) in 100mL of 20mM sodium phosphate buffer (pH 7.0) containing 6.7mM sodium chloride. The colour reagent, a solution containing 96mM 3,5dinitrosalicylic acid (20mL), 5.31 M sodium potassium tartrate in 2M sodium hydroxide (8mL) and de-ionized water (12mL). A serial dilution test was carried with six test tubes each containing 1mL of starch, varying concentration of protein, 1mL of distilled water, 1mL of enzyme solution and 1mL of colour reagent. After the starch, protein, distilled water and enzyme solution were mixed and incubated at 25°C for 30 minutes. Then, 1mL of the colour reagent was added and the stoppered tube was placed into an 85°C water bath. After 15 minutes, the reaction mixture was removed from the water bath and cooled thereafter, and the absorbance value determined at 540nm.

Percentage inhibition activity is calculated using;

$$\% \text{ inhibition} = \frac{A_c - A_s}{A_c} \times 100$$

Where A_c = absorbance of control
 A_s =absorbance of substrate.

2.5.1 Effect of pH on α - amylase inhibitory activity

Optimal pH of α - amylase activity was determined using the pH range of 7.0-11.0, at an interval of 0.5; this was done by adjusting the pH concentrations with 0.1M NaOH and 1N of HCl. The enzyme activity was assayed by adding 0.5mL of starch, 0.5mL of α - amylase enzyme in two different test-tubes per each pH in which 0.5mL of protein extract is added into one test-tube and the other contains no protein extract. The absorbance of each pH was determined at 540nm.

2.5.2 Effect of temperature on α - amylase inhibitory activity

The optimum temperature of α - amylases was determined using different temperatures for enzymes reaction (30, 40, 50, 60, 70, 80, 90 and 100°C) with 1mL of starch as substrate, 1mL of the enzyme after the temperature is determined then 1mL of Dinitrosalicylic acid (DNS) to stop the enzyme activity. Absorbance is determined at 540nm.

2.5.3 Effect of salts on α - amylase inhibitory activity

The effect of four metals salt (Na, K, Mn, and Mg) on the inhibitory activity of the protein isolate on α -amylase was observed by incubating varying concentrations of their respective salt solutions (NaCl, KCl, MgSO₄, MnSO₄.H₂O) at 25°C for 30minutes with 0.5mL of starch, 0.5mL of alpha amylase enzyme and 0.5mL of protein isolate. The reaction was stopped by the addition of 1mL of Dinitrosalicylic acid (DNS) reagent and placed in an 85°C water bath. After 15minutes, the test tubes are removed and allowed to cool. Absorbance is determined at 540nm.

2.5.4 Mode of α - amylase inhibition

To determine the Mode of inhibition of α - amylase by the unripe plantain isolate, a modified method of Ali *et al.* [17], the activity of the enzyme was assayed by incubating the enzyme solution at 25°C for 30minutes with varying substrate concentrations (soluble starch) ranging from 0.2 -1.0mL while other components are kept constant. The reaction was stopped by the addition of 1mL of Dinitrosalicylic acid (DNS) reagent and placed in an 85°C water bath. After 15minutes, the test tubes are removed and allowed to cool. Absorbance was measured at 540nm, and the mode of inhibition of the protein isolate on α - amylase was determined by analysis of the double reciprocal graph plot using graph pad prism software.

2.6 Pancreatic Lipase inhibition Assay

The porcine pancreatic lipase inhibitory assay was adapted from Zheng *et al.*, 2010, and Bustanji *et al.*, (2010) [18, 19], with some modifications. A stock solution of PNPB (p-nitrophenyl butyrate) was prepared by dissolving 20.9 mg of PNPB in 2 mL of acetonitrile. 0.1 mL of porcine pancreatic lipase (1 mg/mL) was added to test tubes containing various concentrations of protein isolate. The resulting mixtures were then made up to 1 mL by adding a Tri-HCl solution (pH 7.4) and incubated at 25 °C for 15 min. After the incubation period, 0.1 mL of PNPB solution was then added to each test tube. After the incubation period, 0.1 mL of PNPB solution was then added to each test tube. The mixture was again incubated for 30 min at 37 °C. Pancreatic lipase activity was determined by measuring the hydrolysis of p-nitrophenyl butyrate to p-nitrophenol at 405nm using a UV-visible spectrophotometer.

Percentage inhibition activity is calculated using;

$$\% \text{ inhibition} = \frac{A_c - A_s}{A_c} \times 100$$

Where A_c = absorbance of control

A_s =absorbance of substrate.

2.6.1 Effect of pH on Pancreatic lipase inhibitory activity

The Ideal pH of Pancreatic Lipase activity was determined using the pH range of 7.0-11.0, at an interval of 0.5; this was done by adjusting the pH concentrations with 0.1M NaOH and 1N of HCl. The enzyme activity was assayed by adding 0.5mL of PNPB (p-nitrophenyl butyrate), 0.5mL of pancreatic lipase enzyme in two different test-tubes per each pH in which 0.5mL of protein isolate is added into one test-tube and the other contains no protein isolate. The absorbance of each pH was determined at 540nm.

2.6.2 Effect of temperature on Pancreatic lipase inhibitory activity

The optimum temperature of pancreatic lipase was determined using different temperatures for enzymes reaction (30, 40, 50, 60, 70, 80, 90 and 100°C) with 1mL of PNPB (p-nitrophenyl butyrate) as substrate, 1mL of the enzyme after the temperature is determined. Absorbance is determined at 540nm.

2.6.3 Effect of salts on Pancreatic lipase inhibitory activity

The effect of four metals salt (Na, K, Mn, and Mg) on the inhibitory activity of the protein isolate on pancreatic lipase was observed by incubating varying concentrations of their respective salt solutions (NaCl, KCl, MgSo₄, MnSo₄.H₂O) at 25^oC for 30minutes with 0.5mL of PNPB (p-nitrophenyl butyrate), 0.5mL of Pancreatic Lipase enzyme and 0.5mL of protein isolate While temperature and pH were kept constant. Absorbance is determined at 540nm.

2.6.4 Mode of Pancreatic Lipase inhibition

To determine the Mode of inhibition on Pancreatic Lipase by the unripe plantain isolate, the method described by Ali *et al.* [17] was used with some slight modifications. The mode of inhibition of the enzyme by the protein isolate was determined by incubating the enzyme solution at 25^oC for 30minutes with varying substrate concentrations of “PNPB (p-nitrophenyl butyrate)” ranging from 0.2 -1.0mL while other components are kept constant. Absorbance was measured at 540nm, and the mode of inhibition of the protein isolate on pancreatic lipase was determined by analysis of a double reciprocal graph plot using graph pad prism software.

2.6.5 Statistical analysis

Statistical Analysis was performed using GraphPad Prism 7 statistical package (GraphPad Software, USA) and Microsoft Excel. The data were analyzed by one-way analysis of variance (ANOVA) followed by Bonferroni test. All the results were expressed as Mean±SE for triplicate determinations. Significance was accepted at $P \leq 0.05$.

3. RESULTS

3.1 Inhibitory effects of Unripe plantain protein isolate on α -amylase and pancreatic lipase in vitro

First, the inhibitory effect of unripe plantain protein isolate on α -amylase was investigated by using p-Nitrophenyl- α -D-glucopyranoside as a substrate and was compared to the effect of the clinically applied α -amylase inhibitor, acarbose (Fig.1). The activity of α -amylase was inhibited by unripe plantain protein isolate in a concentration-dependent manner, in a range of (0.2 - 1.0 mg/mL). Amongst all the concentrations, the α -amylase Inhibition tends to be highest at 1.0 mg/mL with a percentage inhibitory activity of 87% followed by 0.8 mg/mL and 0.6 mg/mL with inhibitory activity of 83% and 76% respectively. The mode of enzyme inhibition was uncompetitive (fig.2). Acarbose, a clinically applied α -amylase inhibitor used as an oral hypoglycemic agent, inhibited α -amylase activity by 86% at a concentration of 1.0 mg/mL. These results show that the Unripe plantain protein isolate has a slightly higher α -amylase inhibition potential than acarbose.

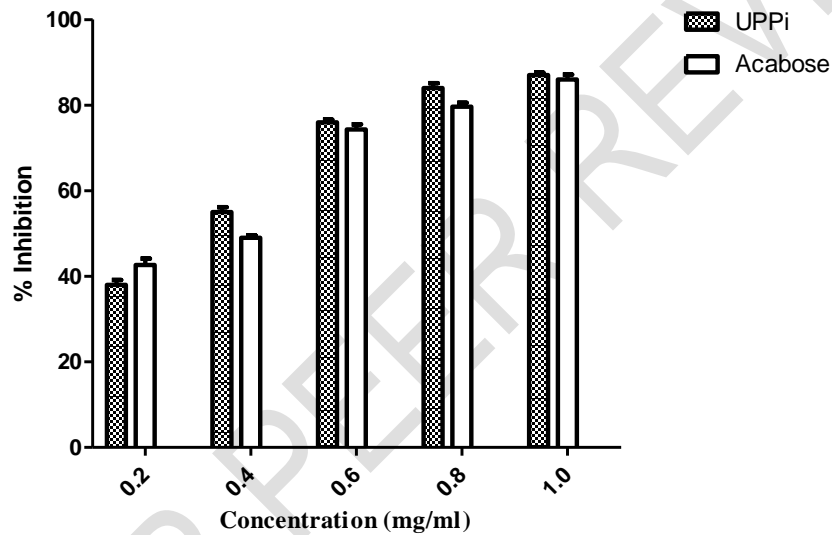


Figure 1: Inhibitory Activities of Unripe Plantain protein isolate (UPPi) and Clinical Inhibitor (Acarbose) on α -Amylase Activities

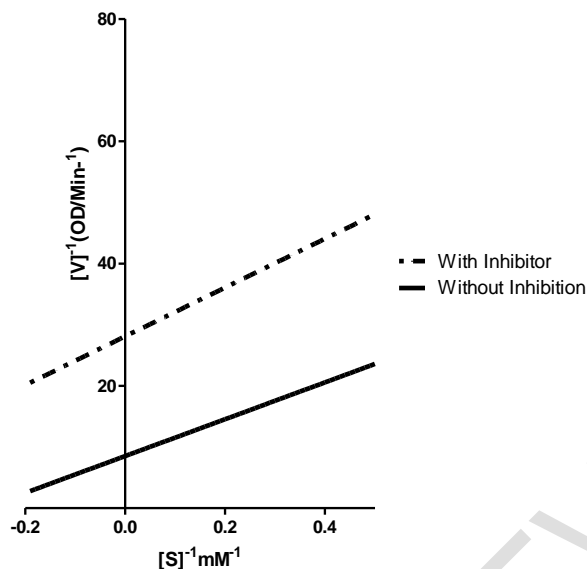


Figure 2: α - amylase mode of inhibition by unripe plantain protein isolate

The inhibitory effects of unripe plantain protein isolate on pancreatic lipase were determined similarly, except p-Nitro phenylbutyrate was used as the substrate. Unripe plantain protein isolates inhibited pancreatic lipase activity in a concentration-dependent in a range of (0.2 - 1.0 mg/mL). Amongst all the concentrations, the pancreatic lipase activity tends to be highest at 1.0 mg/mL with a percentage inhibitory activity of 91% followed by 0.8 mg/mL and 0.6 mg/mL with inhibitory activity of 86% and 65% respectively (Fig.3). The mode of enzyme inhibition was non-competitive (fig.4) Orlistat, a clinically approved drug for obesity treatment, inhibited pancreatic lipase activity by 96% at a concentration of 1.0 mg/mL. This implies that unripe plantain protein isolate has a lower pancreatic lipase inhibition activity than orlistat, however it's still a very effective inhibitor.

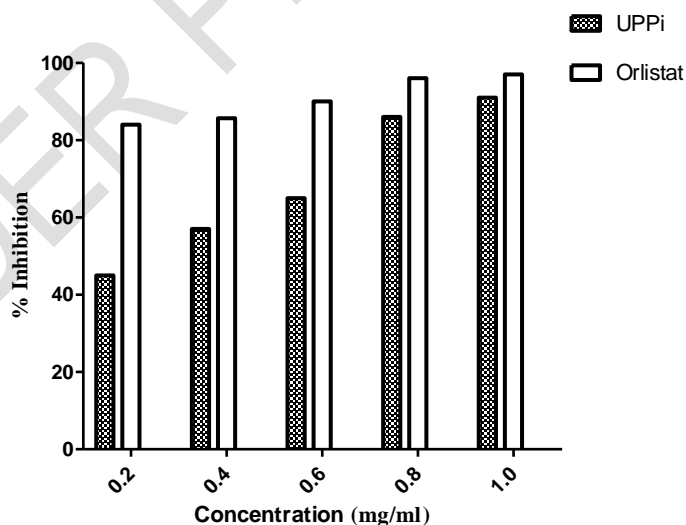


Figure 3: Inhibitory Activities of Unripe Plantain protein isolate (UPPi) and Clinical Inhibitor (Orlistat) on Pancreatic Lipase Activities

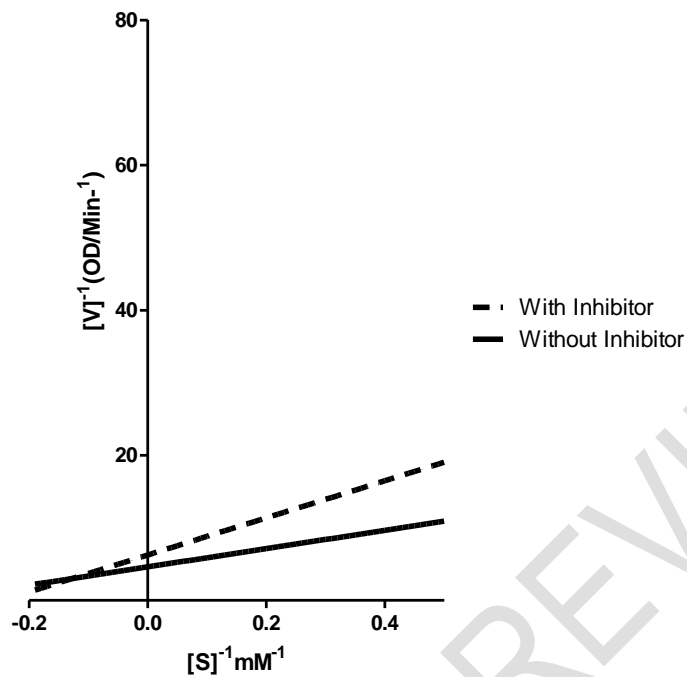


Figure 4: Pancreatic Lipase mode of inhibition by unripe plantain protein isolate

The IC_{50} values of unripe plantain protein isolate for α -amylase and pancreatic lipase were 0.46 ± 1.9 mg/mL and 0.73 ± 3.2 mg/mL, respectively (Table 1) compared to acarbose and orlistat. These results indicate that Unripe plantain could be useful as a natural anti-hyperglycemic and anti-obesity material.

Table 1: This table shows the IC_{50} values of the inhibitory activities of the Unripe plantain (*Musa paradisiaca*) protein isolate on α - Amylase and Pancreatic Lipase

	α - Amylase (mg/mL)	Pancreatic Lipase (mg/mL)
Unripe plantain (<i>Musa paradisiaca</i>) protein isolate	0.46 ± 1.9	0.73 ± 3.2
Acarbose	0.51 ± 3.6	-
Orlistat	-	0.61 ± 9.4

3.2 Effect of pH, and Temperature on enzyme inhibitory activities

Furthermore, the effect of pH, and temperature on α - amylase inhibitory activities were investigated, and the results showed that the optimum pH (Fig.5) and optimum temperature (Fig.6) for α - amylase inhibition was pH 9.0, and 40°C respectively. The Significance of pH, and temperature on pancreatic lipase inhibitory activities were also investigated, and the results also displayed that the optimum pH (Fig.7) and optimum temperature (Fig.8) for pancreatic lipase inhibition were pH 9.0, and 40°C respectively.

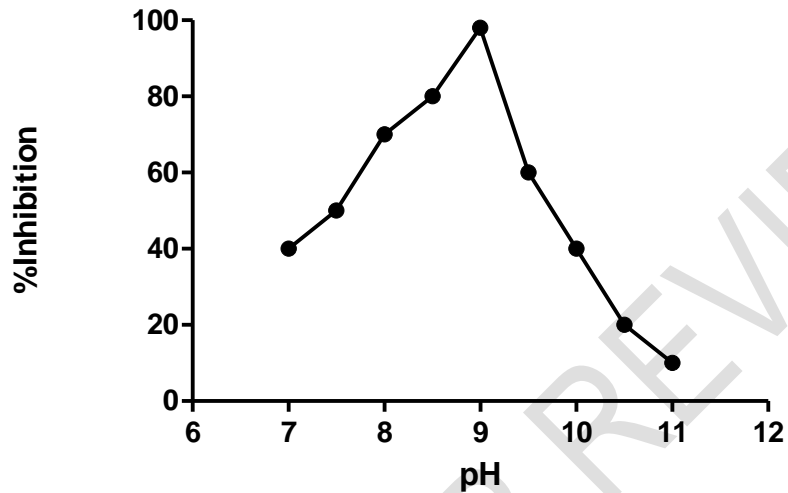


Figure 5: Effects of pH on Inhibitory Actions of Unripe Plantain protein isolate on α - Amylase Activities

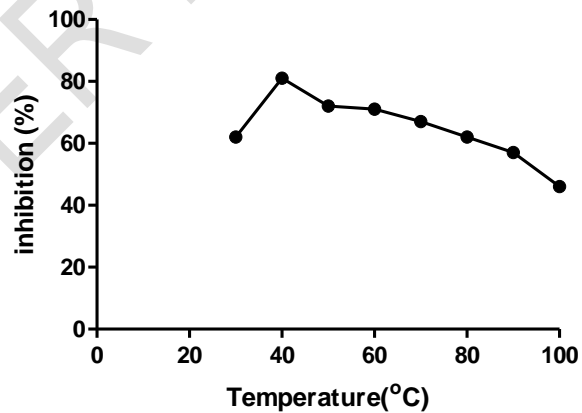


Figure 6: Effects of Temperature on the Inhibitory Actions of Unripe Plantain Protein isolates on α - Amylase

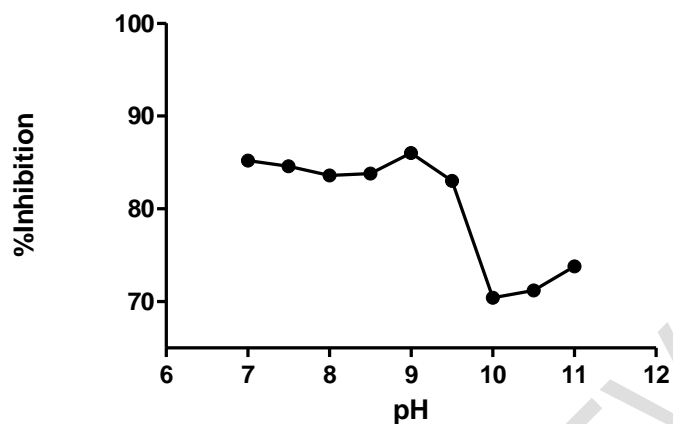


Figure 7: Effects of pH on Inhibitory Actions of Unripe Plantain Protein isolates on Pancreatic Lipase Activities

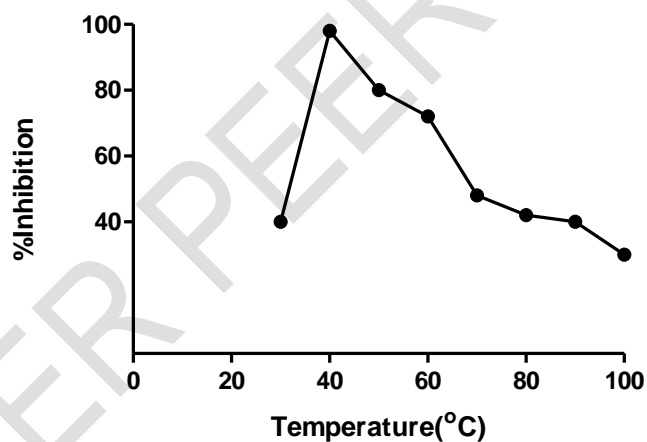


Figure 8: Effects of Temperature on the Inhibitory Actions of Unripe Plantain Protein isolates on Pancreatic Lipase Activities

3.3 Effects of salts on enzyme inhibitory activities

Gibbs and Alli [20], first reported the effect of various salts on enzyme/inhibitor complexation. We determined the effect of salts on α -amylase and pancreatic lipase inhibitory activity by adding varying concentrations (0.1- 0.5 mmol) of NaCl, KCl, MgSO₄, MnSO₄.H₂O to a fixed enzyme and inhibitor concentrations whilst keeping the temperature and pH constant.

The results (fig.9) showed that NaCl, KCl, MgSO₄, MnSO₄.H₂O salts showed an antagonistic effect, reducing the inhibitory potential of unripe plantain Protein isolates on the α -amylase activity at all concentrations.

The (fig.10) indicated that at a concentration of 20mmol, $MnSO_4 \cdot H_2O$, had a synergistic effect on unripe plantain protein isolates inhibition of pancreatic lipase, increasing it from 97% to 100%, while at other concentrations it reduced the inhibitory activities. At a concentration of about 30mmol, KCl, $MgSO_4$, and NaCl did not show any effect on the enzyme's inhibitory activities, while at other concentrations it drastically reduced the inhibitory potential of unripe plantain protein isolates on pancreatic lipase activities.

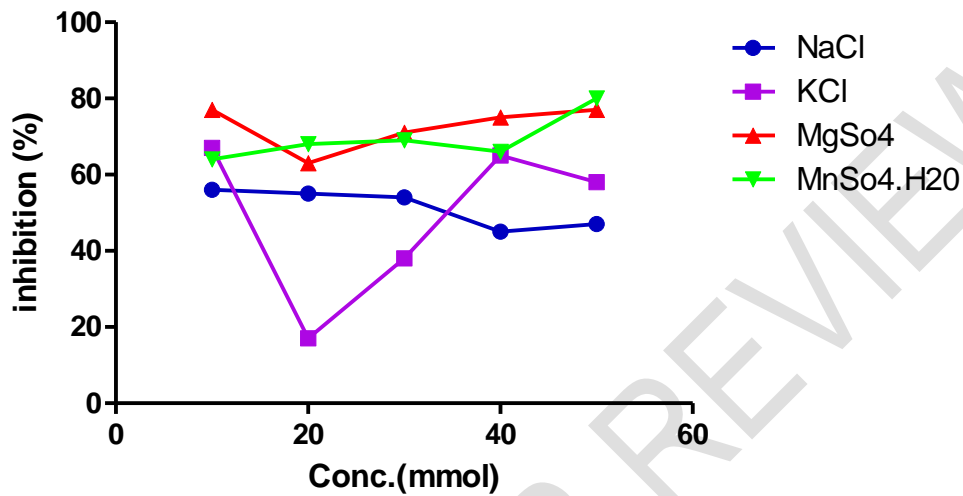


Figure 9: Effects of Salts on the Inhibitory Actions of Unripe Plantain protein isolates on α -Amylase Activities

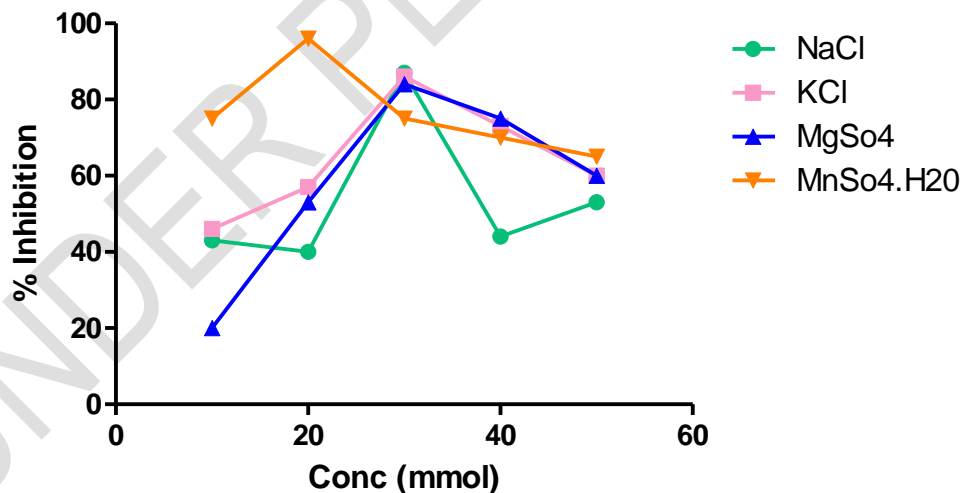


Figure 10: Effects of Salts on the Inhibitory Actions of Unripe Plantain protein isolates on Pancreatic Lipase Activities

4.DISCUSSION

Alpha amylase inhibitors are known as starch blockers because they contain constituents that stop dietary starch from being absorbed by the body. Thus α - amylase is useful in the treatment of diabetes mellitus and its several complications. they exert their blood glucose-lowering effect through the inhibition of enzymes such as salivary and pancreatic amylase [21]. It has been suggested by Kazeem *et al.* [22] that α - amylase inhibitors are more effective therapeutic agents for the control of postprandial hyperglycaemia thus unripe plantain isolates could be an effective therapeutic agent in the management of diabetes mellitus and the results of our study confirmed that.

Lipid metabolism is smartly balanced to maintain homeostasis. When this balance is lost, obesity or hyperlipidaemia develops, resulting to a diversity of serious diseases, including hypertension, diabetes, atherosclerosis and reduction in the functionalities of certain organs. Therefore, the management of lipid metabolism by drugs could be used to prevent or treat these diseases. Pancreatic Lipase inhibition is one of the most widely studied mechanisms for the determination of the possible efficacy of natural products as anti-obesity agents. Acarbose and Orlistat, two clinically permitted medications for diabetes and obesity treatment, has been revealed to act through inhibition of α - Amylase and Pancreatic Lipase respectively. Although they are one of the best-selling medications worldwide, it has some undesirable gastrointestinal side effects like flatulence, oily spotting and stools among others. The success of orlistat and acarbose has driven research for the identification of different α -amylase and pancreatic Lipase inhibitors that lack some of these unpleasant side effects. Pancreatic lipase and α - amylase Inhibitors from plants constituents identified from indigenous medicinal plants present an exciting opportunity for the development of newer therapeutics. As part of the continuing search for bioactive anti-obesity and antidiabetic agents from natural herbal resources, various plants have been screened for their anti-lipase and anti-diabetic activity [5].

4. CONCLUSION

This study showed that the potential anti-diabetic and anti-obesity actions of unripe plantain is via its inhibitory effect on α -amylase and pancreatic lipase. Furthermore, unripe plantain can help the suppression of increased postprandial blood glucose levels. Thus, we suggest that unripe plantain could be added to the diets of patients with diabetes.

REFERENCES

1. Ng, M., et al., *Global, regional, and national prevalence of overweight and obesity in children and adults during 1980–2013: a systematic analysis for the Global Burden of Disease Study 2013*. The lancet, 2014. **384**(9945): p. 766-781.
2. Astrup, A. and N. Finer, *Redefining type 2 diabetes: 'diabesity' or 'obesity dependent diabetes mellitus'?* obesity reviews, 2000. **1**(2): p. 57-59.
3. Hu, F.B., *Globalization of diabetes: the role of diet, lifestyle, and genes*. Diabetes care, 2011. **34**(6): p. 1249-1257.
4. Alberti, K.G.M.M., P. Zimmet, and J. Shaw, *International Diabetes Federation: a consensus on Type 2 diabetes prevention*. Diabetic Medicine, 2007. **24**(5): p. 451-463.
5. Birari, R.B. and K.K. Bhutani, *Pancreatic lipase inhibitors from natural sources: unexplored potential*. Drug discovery today, 2007. **12**(19-20): p. 879-889.
6. Tiss, A., et al., *Inhibition of human pancreatic lipase by tetrahydrolipstatin: further kinetic studies showing its reversibility*. Journal of Molecular Catalysis B: Enzymatic, 2009. **58**(1-4): p. 41-47.
7. Mahomoodally, M.F., et al., *Traditional medicinal herbs and food plants have the potential to inhibit key carbohydrate hydrolyzing enzymes in vitro and reduce postprandial blood glucose peaks in vivo*. The Scientific World Journal, 2012. **2012**.

8. Olaokun, O.O., et al., *Evaluation of the inhibition of carbohydrate hydrolysing enzymes, antioxidant activity and polyphenolic content of extracts of ten African Ficus species (Moraceae) used traditionally to treat diabetes*. BMC Complementary and Alternative medicine, 2013. **13**(1): p. 94.
9. Truscheit, E., et al., *Chemistry and biochemistry of microbial α -glucosidase inhibitors*. Angewandte Chemie International Edition in English, 1981. **20**(9): p. 744-761.
10. Baron, A.D., *Postprandial hyperglycaemia and α -glucosidase inhibitors*. Diabetes Research and Clinical Practice, 1998. **40**: p. S51-S55.
11. Chiasson, J.-L., et al., *Acarbose for prevention of type 2 diabetes mellitus: the STOP-NIDDM randomised trial*. The Lancet, 2002. **359**(9323): p. 2072-2077.
12. Meena, A.K., P. Bansal, and S. Kumar, *Plants-herbal wealth as a potential source of ayurvedic drugs*. Asian Journal of Traditional Medicines, 2009. **4**(4): p. 152-170.
13. Ghani, A., *Medicinal plants of Bangladesh: chemical constituents and uses*. 1998: Asiatic society of Bangladesh.
14. Willett, W., J. Manson, and S. Liu, *Glycemic index, glycemic load, and risk of type 2 diabetes*. The American journal of clinical nutrition, 2002. **76**(1): p. 274S-280S.
15. Shodehinde, S. and G. Oboh, *Phenolic Extracts of 'Amala' from Unripe Plantain (Musa paradisiaca) Pulps Inhibit Key Enzymes Linked to Type-2-Diabetes and Hypertension*. FUTA Journal of Research in Sciences, 2013. **9**(1): p. 123-134.
16. McCue, P.P. and K. Shetty, *Inhibitory effects of rosmarinic acid extracts on porcine pancreatic amylase in vitro*. Asia Pacific Journal of Clinical Nutrition, 2004. **13**(1).
17. Ali, H., P. Houghton, and A. Soumyanath, *α -Amylase inhibitory activity of some Malaysian plants used to treat diabetes; with particular reference to Phyllanthus amarus*. Journal of ethnopharmacology, 2006. **107**(3): p. 449-455.
18. Bustanji, Y., et al., *Inhibition of hormone sensitive lipase and pancreatic lipase by Rosmarinus officinalis extract and selected phenolic constituents*. J Med Plants Res, 2010. **4**(21): p. 2235-42.
19. Zheng, C.-D., et al., *Screening for anti-lipase properties of 37 traditional Chinese medicinal herbs*. Journal of the Chinese Medical Association, 2010. **73**(6): p. 319-324.
20. Gibbs, B.F. and I. Alli, *Characterization of a purified α -amylase inhibitor from white kidney beans (Phaseolus vulgaris)*. Food Research International, 1998. **31**(3): p. 217-225.
21. Frantz, S., et al., *Repetitive postprandial hyperglycemia increases cardiac ischemia/reperfusion injury: prevention by the α -glucosidase inhibitor acarbose*. The FASEB journal, 2005. **19**(6): p. 591-593.
22. Kazeem, M., et al., *Comparative study on the α -amylase and α -glucosidase inhibitory potential of different extracts of Blighia sapida Koenig*. American Journal of Research Communication, 2013. **1**(7): p. 178-192.