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Article

Investigating the inhibition of catabolic enzymes for implications in cardiovascular diseases and diabetes

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SUMMARY

Non-communicable diseases (NCDs) are chronic health conditions characterized by their prolonged duration and non-infectious nature. These diseases, such as cardiovascular disease (CVD) and diabetes, are often influenced by a complex interplay of genetic, dietary, and lifestyle factors. Obesity, a major risk factor for both diabetes and CVDs, has been linked to excess consumption of carbohydrates and lipids. The enzymes α-amylase and lipase play key roles in the digestion and metabolism of carbohydrates and lipids, respectively. We hypothesized that four different natural substances, ginger, Aloe vera, lemon, and mint leaves, could inhibit the activity of α-amylase and lipase as a potential strategy to mitigate the risk of developing NCDs associated with obesity, CVD, and diabetes. Experimental model as follows: four natural substances were prepared at different concentrations. Solutions containing the enzymes amylase and lipase mixed with their respective buffers, were also made. The polyphenol solutions were then tested for their ability to inhibit the activity of α-amylase and lipase enzymes on their respective substances. Absorbance measurements were taken, and the percentage of enzyme inhibition was calculated for each natural remedy solution at varying concentrations. Experimental data showed that Aloe vera was more effective at inhibiting α-amylase, while ginger had the greatest inhibitory effect on lipase. Data also suggested a higher concentration of these natural substances had a higher inhibitory effect on both α-amylase and lipase enzymes. This study provides insights into natural alternatives that could potentially aid in the management and prevention of NCDs by targeting key enzymes involved in the metabolism of carbohydrates and lipids.

INTRODUCTION

Non-communicable diseases (NCDs) are a major global health burden, with increasing incidence and prevalence worldwide. NCDs are chronic conditions that are not transmissible from person to person but are the result of complex interactions between genetic, environmental, and lifestyle factors (1). According to the World Health Organization, NCDs are responsible for more than 70% of all deaths globally and disproportionately impact low- and middle-income countries. The burden of NCDs is expected to continue to grow in the coming years unless effective prevention and control measures are implemented (1). Cardiovascular diseases (CVDs) and diabetes are two major types of NCDs that have a significant impact on global health. It is estimated that around 75% of individuals worldwide are affected by CVDs, while approximately 11% suffer from diabetes (2). Both conditions can have serious consequences if left untreated. One of the major risk factors for CVDs and diabetes is obesity. The prevalence of obesity has increased dramatically in recent decades with more than 1.9 billion adults worldwide classified as overweight or obese (3). Obesity can lead to significant changes in several physiological processes such as high blood pressure, abnormal blood lipid levels, impaired glucose metabolism, and systemic inflammation (4). The combination of these changes can accelerate the development of conditions such as diabetes and CVDs like atherosclerosis (5). Currently, only a limited number of drugs have received FDA approval for the treatment of obesity. While medications are available to aid weight loss, they often come with unwanted side effects, prompting an increasing interest in exploring natural alternatives with fewer potential risks.

Carbohydrates are an essential macronutrient that serve as a primary energy source, and they are classified based on the number of sugar molecules (6). Excessive carbohydrate consumption may result in weight gain. Lipids perform numerous functions, including energy production and structural support of cells. (7). Fats consist of fatty acids and glycerol, which form triglycerides, the most prevalent dietary lipids. Lipids modulate immune cell plasticity and can induce inflammation in obesity, contributing to associated conditions like CVDs (8). The enzymes α-amylase and lipase play crucial roles in the digestion and metabolism of carbohydrates and lipids, respectively. α-amylase catalyzes the breakdown of starch and complex carbohydrates into smaller sugars, facilitating their absorption and utilization by the body (9). Lipase, on the other hand, catalyzes the hydrolysis of lipids, breaking them down into fatty acids and glycerol, which can then be absorbed and metabolized for energy or stored as adipose tissue (7,10).

Considering this information, some alternative natural substances have been studied for their potential to inhibit enzymes that break down macromolecules such as carbohydrates and lipids, with the goal of decreasing the risk of diabetes, CVDs, and other NCDs. We decided to focus on the effects of ginger, Aloe vera, lemons, and mint leaves because they contain various bioactive compounds such as flavonoids and tannins that may have potential health benefits (11,13).

Ginger is rich in potent antioxidants like gingerols and shogaols, which may protect against oxidative stress and reduce NCD risk (11). Aloe vera leaves contain flavonoids,

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vitamins, alkaloids, glycoproteins, aloe-emodin, and aloin that have been shown to help regulate blood sugar and lipid levels in diabetes (12,13). Lemons provide antioxidants such as vitamin C and flavonoids that combat cellular damage from free radicals (14). Mint leaves (*Mentha Longifolia*) exhibit antimicrobial, digestive, and nervine effects, demonstrating efficacy against certain bacterial strains (15,16).

While natural substances like ginger, Aloe vera, lemon, and mint are known for their potential health benefits, their specific effects on enzymes involved in carbohydrate and lipid metabolism have remained unexplored. Thus, the hypotheses for this project are: Although each one of the natural substances will inhibit the α-amylase and lipase enzymes to some degree, Aloe vera will demonstrate the strongest inhibitory effect against both enzymes. Secondly, the inhibitory effects of the natural substances on α-amylase and lipase will exhibit a dose-dependent response, with higher concentrations demonstrating greater enzyme inhibition. To test our hypotheses, we prepared solutions of the natural substances at varying concentrations and mixed them with α-amylase, lipase, and their respective substances and buffers. Using spectrophotometric analysis, we measured the residual carbohydrate and lipid content to determine the level of enzyme inhibition. Our results showed that Aloe vera was most effective at inhibiting α-amylase, while ginger exhibited the strongest inhibitory effect on lipase. Importantly, we observed a dose-dependent response, where higher concentrations of these natural substances led to greater inhibition of both enzymes. These findings suggest that natural alternatives like ginger and Aloe vera could potentially aid in managing and preventing non-communicable diseases associated with obesity, cardiovascular diseases, and diabetes by targeting key enzymes involved in carbohydrate and lipid metabolism.

RESULTS

This study investigated the inhibitory effects of four natural substances (lemon, ginger, Aloe vera, and mint leaves) on the enzymes lipase and α-amylase, which play crucial roles in the metabolism of lipids and carbohydrates. Modulating the activities of these enzymes through natural inhibitors could present a promising strategy for managing obesity and associated NCDs. To evaluate their efficacy, we tested the natural substances across a range of concentrations to assess potential dose-dependent inhibitory effects on lipase and α-amylase enzymes. Solutions containing the natural substances at different concentrations were mixed with α-amylase and lipase enzymes, along with their respective substances and buffers. Spectrophotometric analysis was then employed to measure the residual carbohydrate and lipid content, which indicated the level of enzyme inhibition by the natural substances. The average percentage inhibition of lipase by various compounds when exposed to different concentrations of natural remedies was calculated **(Figure 1)**. In this experiment, we aimed to investigate substances that could inhibit the lipase enzyme to prevent the breakdown of lipids. For a positive control we measured the lipid content then only the lipid substance without any enzymes present, mimicking complete inhibition where no lipid breakdown could occur. The positive control represented 100% inhibition of lipase activity and had an average absorbance of 3.13. The negative control, which allowed 0% inhibition with full lipase activity, contained only the enzyme and lipid without any inhibitors. The absorbance value of the positive control was then utilized in the percent inhibition equation to calculate the percentage of enzyme inhibition exhibited by each of the natural substances tested. By comparing the absorbance readings of the test samples to the positive control representing 100% inhibition, we quantified the extent to which each natural substance inhibited lipase. All remedies exhibited statistically significant inhibitory effects (p-value<0.05) in a dose-dependent manner. Ginger demonstrated the most potent inhibition of lipase at 70.85%, followed by Aloe vera at 68.31%, lemon at 67.71%, and mint leaves at 64.13% **(Figure 1)**. As the concentration of the natural substances increased, their ability to inhibit lipase also increased.

The average percent inhibition of α-amylase per concentration of the natural substances was calculated **(Figure 2)**. The high positive control average absorbance of 3.13 represented the absorbance value when there was no possibility of carbohydrate breakdown. The absorbance value of the positive control was utilized in the percent inhibition equation to calculate the percentage of enzyme inhibition exhibited by each of the natural substances. The experimental data indicate that the highest concentration of Aloe vera resulted in an average of 82.02% inhibition of α-amylase, while the highest concentrations of lemon, ginger, and mint leaves resulted in 78.66%, 79.66%, and 79.65% inhibition, respectively **(Figure 2)**. At various concentrations, the natural substances tested had different levels of inhibitory effects on α-amylase. Aloe vera had lower inhibitory effects at concentrations of 2%, 4%, and 6% compared to the other substances. However, at a concentration of 8%, Aloe vera had the highest inhibitory effect, while ginger had the highest inhibitory effect at all other concentrations. Therefore, as the concentration of the natural substance increases, the greater the inhibitory effect on α-amylase.

DISCUSSION

The central question of this study was to investigate the inhibitory effects of natural substances like Aloe vera and ginger on the enzymes α-amylase and lipase. We hypothesized that these natural substances would exhibit inhibitory activity against these two enzymes due to their

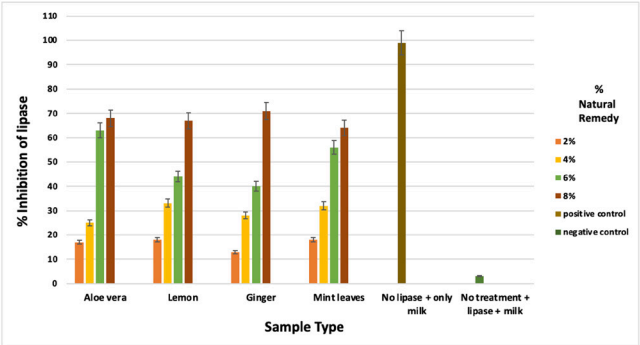

Figure 1: The percent inhibition of lipase measured at varying concentrations. Three independent trials were conducted at each concentration to assess consistency, and the average percent inhibition was calculated. The figure represents the mean percent inhibition values normalized to the average of the positive control readings. Error bars = SEM across the three trials. **p<0.01.

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bioactive compounds. The experimental data showed Aloe vera was most effective at inhibiting α-amylase, while ginger displayed the greatest inhibitory effect on lipase. The higher concentrations of natural substances had the most potent inhibitory effects on both enzymes. For example, the 8% Aloe vera was found to be the most effective at inhibiting both α-amylase and lipase. The diverse range of bioactive compounds present in these natural substances could be interacting with the enzymes through different mechanisms, leading to the observed differences in inhibitory potency. For example, ginger contains compounds like gingerol and shogaols that are more effective inhibitors of the lipase enzyme compared to the citrus flavonoids found in lemon (11). The findings from these experiments demonstrating the inhibitory effects of Aloe Vera and ginger on α-amylase and lipase are particularly noteworthy when compared to synthetic drugs and other treatments targeting these enzymes. For example, Acarbose and Miglitol are synthetic α-glucosidase inhibitors used in the treatment of type 2 diabetes mellitus. While these drugs can effectively reduce postprandial glucose levels, they are often associated with gastrointestinal side effects such as flatulence, diarrhea, and abdominal discomfort (18). On the other hand, natural inhibitors like Aloe Vera and ginger may offer a safer alternative with fewer adverse effects.

Experimental errors are possible, primarily due to the nature of the experiment. One possible error in this experiment is the contamination of the cuvettes by microscopic particles which can alter the absorbance measured by the spectrophotometer. This can lead to inaccurate calculation of results. By regularly cleaning and sterilizing the cuvettes with Kim Wipes and distilled (DI) water before use, this was minimized as much as possible. Another possible error is temperature fluctuations within the water bath incubator. The temperature of the water bath may not have been stable throughout each trial, which could have resulted in an inaccurate reading of the absorbance due to factors such as ambient air exposure or lack of temperature monitoring, as temperature fluctuations can alter enzymatic reaction rates and consequently affect absorbance readings. This error could be minimized by using a laboratory incubator with reliable temperature control to ensure it is within the desired range and consistent with all the samples.

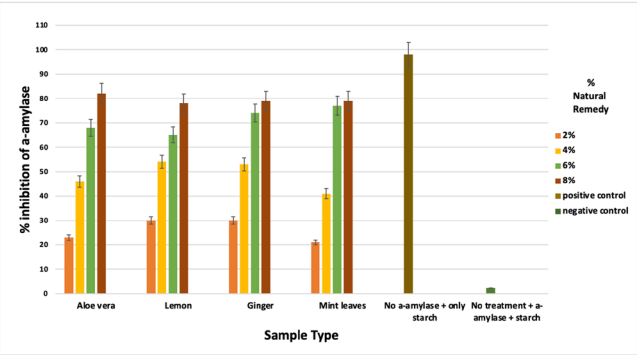

Figure 2: The percent inhibition of α-amylase measured at varying concentrations. Three independent trials were conducted at each concentration to assess consistency, and the average percent inhibition was calculated. The figure represents the mean percent inhibition values normalized to the average of the positive control readings. Error bars = SEM across the three trials. ** p<0.01.

Obesity is a major risk factor for developing NDCs like cardiovascular diseases and diabetes. By investigating natural inhibitors of enzymes involved in carbohydrate and lipid metabolism, such as α-amylase and lipase, this study could provide insights into potential strategies for managing obesity, by potentially leading to a natural way to decrease the absorption of calories from food. Reducing obesity may subsequently help mitigate the incidence of associated conditions like cardiovascular diseases and diabetes, which are major public health problems affecting millions worldwide. These conditions can have serious consequences, including disability and death, and they also have significant economic costs, as they require ongoing medical treatment and can lead to lost productivity. Nowadays, the worldwide scientific community is looking for alternative natural therapies that have fewer side effects and that may be more widely available and affordable. This experiment provides valuable insights into the inhibitory effects of natural substances on specific enzymes involved in carbohydrate and lipid metabolism. However, the translation of these findings to human disease treatment requires further research and validation. While enzyme inhibition is a promising therapeutic approach, the *in vitro* nature of this study does not directly translate to the complex physiological conditions present in NCDs. To bridge this gap and evaluate the potential clinical relevance of these natural substances, future studies could involve using *in vivo* animal models and, ultimately, clinical trials. .

MATERIALS AND METHODS

Preparation of natural substance solutions

Firstly, all the materials were properly cleaned and sterilized with distilled water before use. Lemon, ginger, Aloe vera, and mint leaf solutions were prepared at 2%, 4%, 6%, and 8% concentrations. For lemon, the juice was extracted using a reamer and mixed with distilled water. To obtain ginger juice, the ginger was grated, and the juice strained through a mesh. For mint leaves, the leaves were blended with distilled water and then the mixture was strained through a mesh to obtain the juice.

Preparation of macromolecule solutions

A 10% (w/v) wheat starch solution was made to serve as the carbohydrate substance for α-amylase. Additionally, 10 mL of whole milk, as it contains lipids that serve as the substance for lipase was obtained. Both these solutions were filtered through grade 1 filter paper.

Experimental setup

To prepare the α-amylase solution, 5 g of α-amylase powder were obtained (Flinn Scientific, A0283) and mixed with 100 mL distilled water. A 0.036 M sodium phosphate buffer solution (Carolina Science, 89-1442) was also measured and mixed. In a beaker, this buffer was combined with the α-amylase mix to create an "amylase buffer solution", which was incubated at 37°C for 5 minutes. For the lipase solution, 5 g of lipase powder (Carolina Science, 87-2500) were obtained and mixed with 100 mL distilled water. A 0.036 M sodium phosphate buffer solution (pH 6.4) was separately prepared. The concentrated lipase solution was then mixed with the sodium phosphate buffer solution in a beaker to create the "lipase buffer solution," which was incubated at 37°C for 5

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minutes. Lastly, 0.5 mL of each substance solution (2%, 4%, 6%, 8%) was mixed with 0.5 mL of the "lipase buffer solution" as well as 0.5 mL of the "lipid solution".

For the testing of α-amylase activity, the positive control was prepared by adding 0.5 mL of the "carbohydrate solution" into a test tube. For the negative control, 0.5 mL of the "carbohydrate solution" was mixed with 0.5 ml of the "amylase buffer solution" in a separate test tube, and 1 mL of the potassium iodide indicator was added to both solutions. For testing of lipase activity, the positive control was prepared by taking 0.5 mL of the "lipid solution" into a test tube. For the negative control, 0.5 ml of the "lipid solution" was mixed with 0.5 mL of the "lipase buffer solution" in a separate test tube. Lastly, 1 mL of 1% phenolphthalein solution was added to the positive and negative control solutions.

Absorbance Measurements

The spectrophotometric assays were conducted using a Spectronic 200 Spectrophotometer to measure absorbance at specific wavelengths. For the α-amylase assay, residual starch was detected with a potassium iodide indicator that forms a blue-purple starch complex, and the absorbance of this complex was measured at 630 nm. For the lipase assay, residual lipids were detected using a phenolphthalein indicator that turns pink in the presence of lipids, with absorbance measured at 550 nm. Cuvettes containing positive and negative controls, as well as varying concentrations (2-8%) of the natural substances mixed with the respective enzyme-substance solutions, were measured in triplicate after a 30 minute incubation at 37°C, were measured after a 30 minute incubation at 37°C. The average percentage enzyme inhibition was calculated from the absorbance readings using the equation:

$$(1 - (\frac{absorbance\ of\ positive\ control\ -\ absorbance\ of\ the\ sample}{absorbance\ of\ positive\ control})) * 100$$

The non-overlapping error bars, set at the standard error, and the results of an analysis of variance (ANOVA) test further showed the statistical significance of the data. The calculated F-statistic was greater than the critical value, with **p<0.01.

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