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Short Communication

Assessment of inhibitory action of tea extract on human salivary amylase

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Tea, an aromatic beverage from *Camellia sinensis,* is the second most consumed drink after water. The different types of tea include black, green, oolong, etc. Salivary amylase is an enzyme that helps digestion by breaking down polysaccharides into smaller molecules, contributing to elevated blood glucose levels and postprandial hyperglycemia. The scientific community is exploring natural inhibitors of amylase to slow down starch digestion and lower postprandial blood glucose levels for diabetes management. Plant-derived α-amylase inhibitors are gaining popularity as safe and cost-effective alternatives. The current study included the effect of tea extract on the action of human salivary amylase. The result demonstrated that the extract slowed down human salivary amylase activity in a dose-dependent manner. At the concentration of 1% tea extract, the achromatic point was reached in an hour, while in the control sample, it was achieved within 5 minutes. In conclusion, tea consumption affects the attributes of human saliva by inhibiting the activity of salivary amylase, thereby affecting the digestion of starch. Thus, tea could potentially be used to manage glucose levels in diabetes patients.

Keywords: Diabetes, Human salivary amylase, Inhibitory action, Tea extract

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Introduction

Starch is typically the main source of digestible carbohydrates in the human diet. It is a polysaccharide found in staple foods like wheat, maize, rice, and potatoes and is a major energy source. Digestion of starch into glucose determines its nutritional quality and affects blood glucose levels, insulin response, and metabolic processes[1]. Rapid digestion of starch can lead to a sudden rise in blood glucose levels, while slow digestion causes a steadier rise in blood glucose levels[2]. The glycemic index (GI) is used to classify carbohydrate digestion and its absorption rates[3]. Rapid starch digestion may cause postprandial hyperglycemia hyperinsulinemia, and high GI, which is associated with an increased risk of type-2 diabetes and obesity-related complications[4].

Salivary amylase is the most abundant enzyme in human saliva, mainly formed in the parotid gland. Its activity varies widely between individuals. Its functions include breaking down polysaccharides and aiding in carbohydrate metabolism[5]. Starch digestion begins in the mouth with salivary α-amylase catalytic activity. It continues in the stomach until the increasing acidity inactivates it (between pH 3-4), and then resumes in the small intestine[6,7]. The salivary α-amylase breaks down amylose and amylopectin by cutting their α-1-4 glycosidic bonds[8].

Inhibiting digestive amylases can slow down starch digestion, thereby decreasing starch-derived glucose levels and improving glycemic responses, which can be useful to manage conditions like type-2 diabetes[9]. Several plant extracts have been reported to inhibit the activity of α-amylase. For example, the extracts of *Eugenia dysenterica*, *Stryphnodendron adstringens*, *Pouteria caimito*, *Pouteria ramiflora*, and *Pouteria torta* exhibited strong α-amylase inhibitory activity[10]. The extracts of *Ficus bengalensis* bark, *Syzygium cumini* seeds, *Cinnamomum verum* leaves, and *Curcuma longa* rhizome showed inhibition of human pancreatic amylase[11].

Tea (*Camellia sinensis*) is one of the most popular beverages worldwide[12]. Based on the processing, it can be classified into black, green, and oolong. The main chemical compositions in tea include polysaccharides, proteins, amino acids, polyphenols, organic acids, alkaloids, and volatile compounds[13,14]. Tea is known to have notable pharmacological activities, such as antioxidant, antidiabetic, and anticarcinogenic effects[15,16].

Combining a starch-rich diet or meals with food items that have an inhibitory effect on amylases could benefit healthy individuals and those with diabetes or challenges in glycemic management[17]. Therefore, the present study aimed to study the inhibitory action of tea extract on human salivary amylase (α-amylase).

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Materials and Methods

Salivary amylase preparation

The research was conducted at the Laboratory of the Department of Zoology, Deshbandhu College, University of Delhi, Delhi, India. Saliva samples were collected from ten healthy volunteers (5 males and 5 females) of age groups between 16 to 22 years with their consent. The volunteers were asked to chew a few chunks of paraffin wax in their mouths for 5 minutes. The saliva mixed with paraffin wax was collected in a beaker. The saliva was filtered with a wet-squeezed cotton filter, appropriately diluted, and kept in a sterilised reagent bottle for experimental purposes.

Tea decoction preparation

Tea decoction was prepared by boiling 10 g of tea leaves (procured from the market during June-July-2023) in 500 mL water for 5 minutes. Tea decoction was then sieved using a stainless-steel strainer to remove impurities. Then, it was filtered through Whatman paper No. 1. Subsequently, different concentrations of this tea decoction, i.e., 0.125, 0.25, 0.5, and 1%, were prepared using double distilled water.

Digestion mixture Preparation

A digestion mixture was prepared by adding 5 mL of 1% starch solution, 2 mL phosphate buffer (pH 6.8), and 2 mL of 1% sodium chloride solution in sterilised borosil test tubes. The digestion mixture was kept in a water bath for 5 minutes at 37°C. A parallel set-up of the digestion mixture was also prepared for the control experiment.

Indicator to detect the enzymatic activities

To detect the enzymatic activities, 2 mL of 1% Potassium Iodide (KI) solution was added into serially labelled sterilised borosil test tubes (indicator tube). Similarly, a series of tubes were prepared for the control experiment. These labelled indicator tubes were kept in the test tube stand to record the inhibition of salivary amylase activity.

Assessment of salivary amylase inhibition

Both test experiment and control experiment setups, which were kept in the water bath for equilibrium, were added 1 mL of the pre-treated enzyme with tea concoction in a 1:1 ratio and mixed well. In the control experiment, added 1 mL of distilled water and mixed well. After the addition of the enzyme, within 5-10 seconds, 100 µL of this digestion mixture was put into the test tubes containing iodine as an indicator. A continuous change in colour in the indicator tube was recorded till the achromic point was not achieved. To get the achromic point, after every minute and in a later stage, after every 30 seconds, 100 µL from the digestion mixture was added into the indicator tubes. The experiment was repeated three times with all the different concentrations, i.e. 0.125, 0.25, 0.5, and 1%, along with the control experiments.

Statistical analysis

The test and control experiments' results were recorded and presented as Mean+SE. (Standard Error). These obtained results were analysed statistically using SPSS version 19.1. One-way ANOVA, followed by Tukay's test, was done to find a statistically significant difference in the experiments at $p<0.05\%$.

Results and Discussion

The salivary amylase in saliva acts on starch and converts it into maltose. Starch gives a blue or dark brownish colour with iodine until completely digested into maltose. At this point, no blue colour appeared. This indicates the endpoint or achromic point.

The results indicate that the tea concoction slowed down the activity of salivary amylase in a dose-dependent manner (Fig. 1). In control, the achromic point was reached in 5 minutes, which is represented by 1/T, and that is equal to 0.2/min. Meanwhile, in the lowest test concentration of tea extract, i.e., 0.125%, the enzymatic activity was recorded at 0.125/min. Similarly, in the digestion mixture containing 1% tea extract, the achromic point was reported after 60

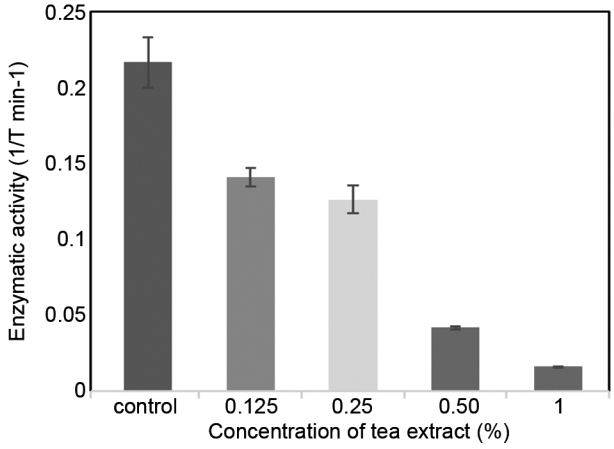

Fig. 1 — The effect of tea extract on human salivary amylase activity.

minutes of incubation, and enzymatic activity was recorded at 0.0167/min (Fig. 1). In test experiments with 0.25 and 0.5% tea concoctions, the achromic point was achieved in 8.34 and 24.67 minutes, and the enzymatic activities were 0.11/min and 0.04/min respectively. A statistically significant difference was obtained between the groups. These results indicate that the tea extract inhibited the enzymatic action of salivary α-amylase in a dose-dependent manner.

The present study is in agreement with the earlier studies conducted on the inhibitory action of black tea and lemon juice on salivary and pancreatic amylases[17]. It has been reported that tea decoctions prepared from black and green teas can inhibit salivary amylase in human saliva[18]. Similarly, bacterial amylase was also inhibited by tea decoctions[18]. Black tea showed higher inhibition levels than green teas, and the removal of tea tannins resulted in the loss of inhibitory activity from all decoctions[18]. Furthermore, studies reported the inhibition of starch digestion by *Camellia sinensis* extract in a dose-dependent manner[19]. Tannin was also reported to possess an inhibitory effect on human salivary α-amylase[20].

The inhibitory effect of tea decoction on salivary amylase activity may be associated with phytoconstituents of *C. sinensis*. Literature mining of phytochemical constituents of *C. sinensis* revealed the presence of flavonoids (rutin and isoquercitrin), which can inhibit salivary α-amylase activities, warranting effective type 2 diabetes therapy[19]. The extracts of *Ficus bengalensis*, *Syzygium cumini*, *Cinnamomum verum*, and *Curcuma longa* exhibited concentration-dependent inhibitory action on the pancreatic amylase. The phytochemical analysis of these plants revealed the presence of alkaloids, proteins, tannins, glycosides, flavonoids, saponins, and steroids as probable inhibitory compounds[11]. Additionally, theaflavins and caffeine in tea extract have been shown to have antidiabetic effects[21], and different types of catechins in *C. sinensis*, such as epigallocatechin-3-gallate, epigallocatechin, epicatechin-3-gallate, and epicatechin have shown anti-carcinogenic activities[15].

It can be concluded that tea extract inhibits salivary α-amylase, thereby reducing the rate of starch digestion, leading to lowered glucose levels. The probable inhibitory action of the tea extract could be due to the presence of phytoconstituents in the tea extract. This ability of tea extract can help lower postprandial glycemic index and, therefore, can potentially be used as an effective therapy for diabetes patients.

Conclusion

A dose-dependent effect of tea extract on lowering the action of human salivary amylase indicates that tea consumption along with the carbohydrate meal can inhibit its activity. This slowdown of the starch digestion by the tea can potentially be used to manage glucose levels in diabetes patients. The present study is in vitro, but to strengthen the findings, further research is needed to explore the effects of tea extracts in human or mouse model to check the glycaemic index.

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Conflicts of interest

The authors declare no conflicts of interest regarding the publication of this paper.

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