

Smoking Modulated Salivary Alpha Amylase Synchronized by Daily Beverages

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Abstract

Background: Smoking carry physical and psychological impacts on individual resulting in serious health concerns, among which the direct impacts of smoking on oral cavity regulated by released contents of salivary glands. **Aims:** We sought to identify the impact of smoking on the released concentration of alpha amylase harmonized by daily beverages. **Methods:** To do so, saliva collected from healthy participants (smokers n=30 and smokeless n=30) and a questionnaire placed to define the number of cigarette smoked and beverages intake on daily bases. **Results:** Smoking (137±25) significantly reduced salivary alpha amylase enzyme activity compared to smokeless (147±19). The activity of salivary alpha amylase were further reduced when smoking combined with beverages, tea and cola reaching down to 51±5 and 4±1, respectively. Moreover, obesity reduced the enzyme activity (43.5±13.6) compared to normal weigh (178±18) in smokers. **Conclusion:** Smoking carry risk of reducing carbohydrate sequential digestion resulting in availability of more carbohydrate for intestinal digestion and absorption sparking absorption rate and thereby enhancing obesity and metabolic derangements.

Keywords: Smoking, Salivary Alpha Amylase, Beverages, Obesity, Tea, Cola.

INTRODUCTION

The direct or indirect inhalation of tobacco smoke is responsible about addictive mood changes due to serious physical and psychological addiction potentials,^[1,2] resulting in health hazard^[3] alongside economic considerations^[4] and environmental pollutions.^[5] These risks are related to the many harmful components present in smoke, namely polycyclic aromatic hydrocarbons, carbon monoxide, irritant compounds, and nicotine.^[6] Collectively, carrying risk factors on vital organs reportedly heart, kidney, gastrointestinal system, respiratory system, and liver.^[2,7-13] One-half of tobacco users is under life-threatening with confirmed annual 8 million deaths including 1.3 million of negative smokers.^[14-16] low- and middle-income countries represents 80% of tobacco users.^[16-18] A survey completed in 2020 has estimated that approximately one-quarter of the world population are tobacco users.^[17,18] Jointly, tobacco smoking negatively impacts national economy with annual global costs of tobacco use of 1 trillion U.S. dollars and 2% of the national gross domestic product (U.S. National Cancer Institute and World Health Organization, 2016).^[19] The saliva is oral fluid which has fundamental role in oral physiology.^[20] Saliva is mainly released by the major

salivary glands (parotid, submandibular and sub lingual) and minor production achieved by minor salivary glands dispersed throughout oral mucosa, lips, and palate.^[21] The synthesis and release by acinar cells in the salivary glands under control of the autonomic sympathetic nervous system regulated by activity of the limbic hypothalamic – pituitary – adrenal axis (cortisol) with abundant release during day and decrease during early morning.^[22]

Salivary alpha amylase enzyme (SAAE) is the salivary enzyme with maximum concentration in the pancreas and the salivary glands.^[23,24] The level is highly elevated in pancreatitis and used as a diagnostic tool for pancreatic diseases. The SAAE catalyse the hydrolysis of internal alpha-1,4 glycoside bonds of starch (in rice and potato) producing maltose and oligosaccharides.^[24] Alpha- Amylase enzyme affected by many factors such as Physical exercise, Age, gendre, temperature, pH, diseases, drugs, and food contents.^[25,26] The present study sought to identify the role of smoking on SAAE and the interacting role of oral beverages.

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MATERIALS AND METHODS

Study sample: A total of 60 male participants were enrolled in the present case control study, out of which 30 participants were smokers and 30 were non-smokers, the age range 19-26 years. The study approved and registered at University of Mosul College of Pharmacy (Iraq) (Ref: CoP 07 on 19.03.2024). A consent form were collected from participants to participate in the present study. Smokers enrolled in the present study were active smokers for more than 3 years. Patients using one of the following medication were excluded from the study:

Hyoscine N-butylbromide, Amitriptyline, Diphenhydramine, Chlorpheniramine, Cyproheptadine, Enterostop (Atropine+Diphenoxylate), Librax (Cnidium bromide +Chlordiazepoxide), Co-Codamol (Codeine+Paracetamol), Kanagesic (Orphenadrine +Paracetamol).^[27]

Saliva collection: Saliva collected, from at least 2 hours food-starved participants, by spitting or drooling method by participants into a collection tubes. Participants were asked to rinse mouth to avoid contaminants with food, swallow all saliva and then collected newly secreted saliva in exactly similar period of time (2-5 minutes) for all participants. Collected saliva centrifuged (4000g for 10 minutes) to avoid cells and cell debris. The supernatants were collected for AAE measurement.

The measurement of AAE is conducted by kit supplied

by Biolabo (France), following manufacturer instructions the AAE measured using saliva sample and the resulted outcome colour measured spectrophotometric analysis. The principle of assay based on reacting a known concentration of starch with saliva sample which digest starch and the remaining starch were reacted with iodine to give blue color measured spectrophotometrically, then concentration can be determined from quantification of remaining and hydrolyzed starch.

RESULTS

Data analysis of the present study has confirmed that smoking has impacted the AAE concomitantly with other beverages. BMI has significantly ($p<0.05$) affected the AAE in smokers or non-smokers, however, smoking has further significantly reduced AAE. Tea intake has significantly ($p<0.05$) affected the AAE in smokers or non-smokers, however, smoking has further significantly ($p<0.05$) reduced AAE, and the more tea intake, the more will be the reduction in SAAE. Cola intake has significantly ($p<0.05$) affected the AAE in smokers or non-smokers, however, smoking has further significantly ($p<0.05$) reduced AAE. Surprisingly, when cola intake doubled, the SAAE reduced to their lowest level regardless of smoking. When data of all patients gathered, smoking has significantly ($p<0.05$) reduced AAE when compared to non-smokers (Table 1).

Table 1: Beverages and Smoking Impact on Salivary AAE Activity (U/ml).

Parameters		Non-smokers		Smokers	
		n	Enzymatic Activity (U/ml)	n	Enzymatic Activity (U/ml)
BMI	<25	14	186±22* [^]	18	178±18 [^]
	≥25	16	77±6*	12	43.5±13.6
Tea	0-3	17	150±20* [^]	19	135±30 [^]
	≥4	13	139±18*	11	51±5
Cola	0-1	20	137±17* [^]	21	75±11 [^]
	≥2	10	10±1*	9	4±1
All patients		30	147±19*	30	137±25

Data expressed as Mean±SD, *[^]indicate significantly higher at $p<0.05$ using independent sample t test, *comparison between smokers versus non-smokers, [^]comparison between the same group.

DISCUSSION

Smoking has reduced saliva AAE detrimentally with or without other parameters, however, other parameters have also further contributed to reduce salivary AAE. The impact of beverages were further confirmed as indicated by increasing beverages decrease salivary AEE whether in smokers on non-smokers, but smokers were more impacted.

Smoking has reduced salivary AAE in general and this fact has been previously confirmed by Weiner *et al.*^[28] and explained in the context that aldehyde present in the smoke induces structural changes of AAE via binding of aldehyde with thiol compound of the sAAE.^[28] Surprisingly, a single cigarette smoking has reduced AAE in a study conducted by Zappacosta *et al.*^[29], with similar explanation of cigarette smoke on enzymatic structural modification. In contrast, Jebiril *et al.*^[30] have confirmed

that smoking increased sAAE, however, the increment were only noticed in the young age group (15-30 years) with non-significant differences existed between smokers and smokeless in older age group (31-70 years), resulting in controversial outcomes. Jebiril *et al.*^[30] have explained in the context of reduced salivary flow with older ages. The body weight negatively impact sAAE in the present study, which do harmonized with a study conducted by Aldossari *et al.*^[31] in Saudia Arabia who have found that obesity reduced sAAE regardless of smoking. A parallel observation was confirmed in Chinese, Japanese, and Finland obese individuals.^[32-34] Despite these confirmation of negative impact of obesity on sAAE, the undelying etiology is yet obscure.^[32,35] Similar results obtained in obese and overweight children and the outcomes were explained in the context of sympathetic activity of stress in these children, which do responsible about reduction

of sAAE in these obese children.^[36,37] However, larger scale study reported no association between obesity and sAAE genes copy, nevertheless, this study were enrolled male and female in their study.^[38] Moreover, long-term follow up study also reported no association between BMI and sAAE activity.^[39]

Tea has reduced the sAAE in smokers and smokeless with reduction being greater when concurrent with smoking. In line with this finding, tea inhibits AAE activity and modulate starch digestion reciprocally in previous studies.^[40-42] Tea contains polyphenol which has been confirmed to inhibit enzymatic activity of sAAE via direct binding with enzyme structure leading to protein structural modification.^[43-45] This enzyme inhibition takes place regardless of type or source of tea.^[46,47] However, daily tea or coffee consumption has been reported to induce a steady enzymatic inhibition effects, in which no further reduction of sAAE achieved with additional doses of caffeine.^[46]

In addition to aforementioned impact of caffeine to sAAE. Cola content of caffeine produce same effect and the sweeteners, phosphoric acid, and sugar have additional impact.^[48] In addition, cola contents affect sAAE indirectly through their impact on mouth pH^[49] and the induced histopathological changes.^[50] These collectively confirmed that smoking impact negatively on sAAE, an impact which has been substantiated by consumption of caffeine containing beverages.

CONCLUSION

The popularity of coupling smoking with beverages intake potentially impact salivary alpha amylase levels halting carbohydrate digestion and ultimately impacting weight gain. Advice directed toward those subjects with metabolic diseases, such as, diabetes, obesity, and metabolic syndrome to refrain from smoking at all or at least avoid coupling beverages with smoking. Further researches recommended to establish a certainty of the role of smoking and beverages on other oral proteins or enzymes.

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