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RESEARCH ARTICLE

BIOCHEMICAL ANALYSIS OF SALIVARY TOTAL PROTEIN, ALBUMIN AND α-AMYLASE AMONG THE COFFEE CONSUMED PEOPLE

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ABSTRACT

The quantity and quality of the salivary total protein, albumin, and α-amylase was estimated among the 25 healthy people. The salivary components were estimated before and after coffee consumption. The albumin and α-amylase are decreased after coffee consumption but the total protein was increased after coffee consumption.

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INTRODUCTION

Caffeine (1,3,7-trimethylxanthine) is a natural alkaloid found in coffee beans, tea leaves, cocoa beans, cola nuts and other plants. It is probably the most frequently ingested pharmacologically active substance in the world, found in common beverages (coffee, tea, soft drinks), products containing cocoa or chocolate, and medications, including headache or pain remedies and over-the-counter stimulants (Murphy and Benjamin 1981, IARC 1991b, Dlugosz and Bracken 1992, Carrillo and Benitez 1996).The possibility that coffee consumption can have adverse effects on human health was assessed based on the results of (primarily) published human studies obtained through a comprehensive literature search. The following potential adverse effects of caffeine on human health were investigated: general toxicity, cardiovascular effects, effects on calcium balance and bone status, behavioural effects in adults and children, carcinogenic potential, genotoxic potential, effect on salivary composition and reproductive effects, including pre-and postnatal development (Nawrot *et al.,* 2003). It should be pointed out that review of some of the epidemiological studies was complicated by one or more methodological issues, such as inadequate measurement of caffeine intake; a lack of consideration of all sources of caffeine intake; a lack of consideration of caffeine intake before study; the lack of distinction made between different types of preparation and different strengths of coffee in most studies; inadequate control for the possible confounding effects of variables such as coffee consumption. In Canada, published values for the average daily intake of caffeine from all sources is about 2.4 $mgkg^{-1}$ body weight (bw) for adults and 1.1 $mgkg^{-1}$bw for children 5–18 years old (Chou, 1992). Recently, Brown *et al.* (2001) reported daily caffeine intakes ranging from 288 to 426mg (equivalent to 4.5–6.5 mg kg^{-1} bw in a 65-kg person) in the adult population (481 men and women aged 30–75 years) residing in southern Ontario, Canada. Elsewhere, mean daily caffeine intake for adults among the general population has been given as approximately 3mg kg^{-1} bw in the USA, 4mg kg^{-1} bw in the UK and 7mg kg^{-1} bw in Denmark. For high-level consumers, daily intakes range from 5 to 15$mgkg^{-1}$ bw. For children, daily caffeine intakes have been given as 1mg kg^{-1} bw in the USA, <3 $mgkg^{-1}$ bw in the UK and <2.5 mg kg^{-1} bw in Denmark (IARC 1991b, Ellison *et al*. 1995, Barone and Roberts 1996, Hughes and Oliveto 1997). The purpose of this study was to evaluate the effect of coffee consumption before and after in the salivary composition.

MATERIALS AND METHODS

Sample collection

Twenty five healthy, non-medicated, young males (Age: 21 ± 3 years; body mass: 55 ± 5kg) participated in the study. Each of the subjects performed a single exercise for 10 minutes, smoke a single cigarette and consume a coffee. For each subject, the two testing session were held on consecutive days between 7.30 am and 12.00 am. The samples were collected before and after the coffee consumption. The procedure for each testing session was the same. Subject began a session by tin sing their mouth thoroughly several times with tap water and then resting quietly for 5 minutes. The saliva was collected behind the closed lips (Narazesh, 1982: spitting method) and expectorated at the end of the each minutes into

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ice-chilled container for 5 minutes. All samples were centrifuged and the supernatant used as a sample for the test.

Estimation of total protein (Biuret Method)

Total protein in saliva was determined using the total protein kit from crest biosystem. The unit was expressed as g/dl. Pipette out into clean dry test tubes labeled as blank (B), standard (S) and test (T). Mixed well and incubated at 37°C or at R.T for 30 min. The total protein in the sample was determined with the help of semi auto analyzer (Gornall *et al.,* 1949 and Layne and Ennis, 1957).

$$\text{mg Protein/ml} = \frac{\text{(mg Protein)}}{\text{(ml Reagent D)}}$$

Estimation of albumin (Bromo Cresol Green Method)

Albumin in saliva was determined using the albumin kit from crest biosystem. The unit was expressed as g/dl. Pipette out into clean dry test tubes labeled as blank (B), Standards (S) and test (T). Mixed well and incubated at R.T for 5min.The Albumin in the sample was determined with the help of semi auto analyzer (Duly *et al.,* 2003). Calculation for albumin the following formula

$$\text{Globulin in g/dl} = \underset{\text{(g/dl)}}{\text{(Total protein)}} - \underset{\text{(g/dl)}}{\text{(albumin)}}$$

Estimation of amylase (Direct Substrate Method)

Amylase activity in saliva was determined using the amylase kit from the crest biosystem. And the amylase activity in saliva was expressed as units per liter. Pipette out into clean dry test tube labeled as test (T). Sample was taken from the 1:100 dilutions. Mixed well and the Amylase activity in the sample was determined with the help of semi auto analyzer (Sampson, *et al.,* 1981 and Bretaudiere *et al.,* 1981).

RESULTS AND DISCUSSION

The quantity and quality of the salivary total protein, albumin, and α-amylase was estimated among the 25 healthy people. The salivary components were estimated before and after coffee consumption, total protein (before 0.4780± 0.2490 and after 1.3570±1.0599). Albumin (0.3110±0.2347, 0.12230±7.587) and α-amylase (1576.50±508.65, 1296.10±275.09). The χ^2 square test were analysed the total protein 2.553, Albumin 2.41 and α-amylase 1.533 and all the value significant at 0.5% levels (Table 1, Fig. 1-2). The coffee consumption is involved in the pathogenesis of several diseases regarding different body systems, mainly cardiovascular and respiration in addition to its local toxic effect in the oral cavity. Coffee increased the total protein level but the same time decreases amylase and albumin concentration (Table 1). Form the study we can understand that the salivary secretion is mainly regulated by sympathetic nervous system and parasympathetic nervous system, the stimulation of either sympathetic nervous system or parasympathetic nervous system which cause changes in salivary composition during coffee consumption. The loss of salivary enzyme and protein activities may be due to various

Table 1: Effect of coffee consumption on salivary total protein, albumin and α- amylase levels in saliva (µ/l)

Component	Time	Mean± Stdevi.	X^2 value	Significant
Protein	Before	0.4780±0.2490	2.553	0.020*
	After	1.3570±1.0599		
Albumin	Before	0.3110±0.2347	2.41	0.027*
	After	0.12230±7.587		
Amylase	Before	1576.50±508.65	1.533	0.043*
	After	1296.10±275.09		

The values are expressed as mean ± standard deviation for 25 healthy persons.
* Significant

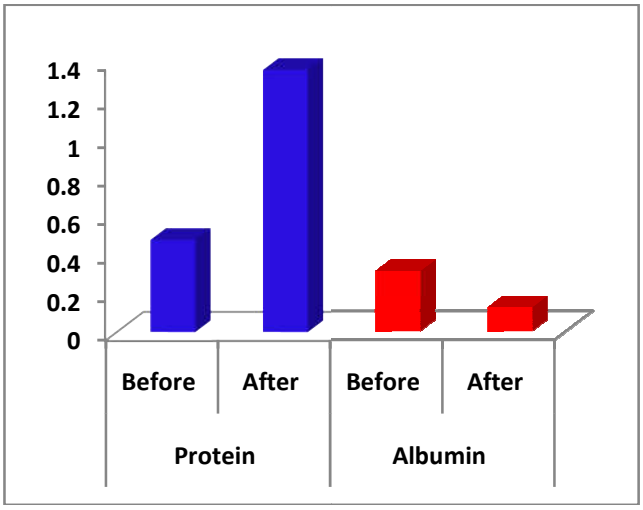

Figure 1: Effect of coffee consumption on salivary total protein and albumin levels in saliva (µ/l)

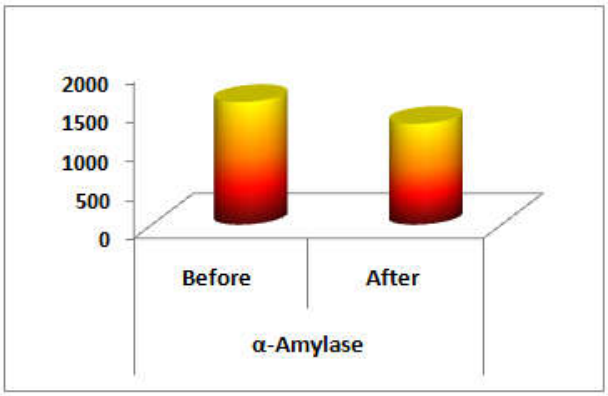

Figure 2: Effect of coffee consumption on salivary α-Amylase levels in saliva (g/dl)

agents in the coffee consumption that affect the enzyme via different mechanisms. The study performed to understand the relationship between salivary components (Protein, albumin and α-amylase). The quantity and quality of saliva secreated depends on the conditions for the entrance through the secreted cells and synthesis in these cells, and on modification as primary saliva passes through the excretory ducts (Suddick *et al*., 1980).These process are regulated in a complex way which includes control by the sympathetic and parasympathetic nerve system (Emmelin, 1981), Neuropeptides (Byod *et al.,* 1991). The mechanism and control of salivary secretions have been reviewed recently (Turner and Sugiya, 2002) Parasympathetic stimulation produces copies saliva low protein concentration while sympathetic stimulation produces little saliva but of high protein concentration and may thus give a sensation of dryness

(Carlson, 2000). In case of coffee consumption both total protein and globulin increased, but the same time the other parameters such as albumin and amylase were decreased after the coffee consumption. The caffeine molecules is structurally similar to adensine, and bind adenosine receptors on the surface of the cells without activating them, This effect called competitive inhibition, interrupts a pathway that normally serves to regulate nerve conduction by suppressing synaptic potentials. The result is an increase in the levels of epinephrine and norepinephrine.Epinephrine, the natural endogrine response to perceived threat, stimulates the sympathetic nervous system. The salivary amylase can be inhibited by the adrenergic blockers present in the coffee. Although studies have frequently speculated that a key aspect of the response to caffeine ingestion is inhibit the amylase secretion (Zhang and Kashket, 1998). Recently Olivia jolly (2006) found that doses of caffenine,200mg increase the amylase secreation,but higher amounts actually produce an apposite effect. The stimulated saliva mostly contained significantly higher proportion of parotion of parotid saliva, but the distribution of the parotid saliva was still extremely variable .These facts are important considering that various areas of the mouth will be exposed to different fluid environment, which may have important implication for the site specificity of several oral diseases.

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