

# The Effect of N-acetylcysteine on Amylase, Electrolytes, Vitamins and Nitrosative Stress Levels in Rats Treated with Maras Powder

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## ABSTRACT

The aim of this study is to investigate the effects of N-acetylcysteine (NAC) on amylase, electrolytes, vitamins and nitrosative stress levels in the plasma of rats treated with smokeless tobacco "maras powder". Our study consisted of three groups. Control (n = 10), the group using maras powder (n = 10), maras powder+ NAC group. To the Maras powder group, 200 mg maras powder was placed in the sublingual area under general anesthesia. It was waited for 15 minutes for Maras powder to be absorbed through mucosa. This procedure was repeated once a day and for 7 days. To the NAC group, 200 mg of Maras powder was given as in the Maras powder group and NAC was injected intraperitoneally with a dose of 100 mg / kg / day. On the 8th day, the levels of amylase, vitamins (A, C and E), electrolytes (Na<sup>+</sup>, K<sup>+</sup>, Cl<sup>-</sup>) and, as the biomarkers of nitrosative stress, nitric oxide (NO) and nitrotyrosine (3-NTx) in the plasma of all three groups were measured by the methods of ELISA. It was determined that amylase, 3-NTx, NO, electrolyte levels increased in the group using Maras powder compared to the control and NAC groups, but vitamin levels decreased. ( $p < 0.05$ ). It was detected that the levels of amylase, 3-NTx, NO, electrolyte in the group treated with NAC decreased compared to the group using Maras powder, whereas vitamin levels significantly increased and approached to the values of the control group. Smokeless tobacco 'Maras powder' increases nitrosative stress and distorts the levels of vitamin A, E, C and electrolyte in the plasma. NAC has a positive function in reducing the damage caused by Maras powder.

**Key Words:** Smokeless tobacco, Maras powder, NAC, vitamins, electrolytes

## Introduction

"Maras powder", which is widely used in Turkey's southern and southeastern regions, is a smokeless form of tobacco. It is made from the leaves of *Nicotina rustina L.* which are dried, pulverized and then mixed with wood ash from oak, walnut and vine grapes. The moistened powder is applied to the labial sulcus for a changing period of between 5 minutes to hours and repeated several times a day. Although it is sometimes used as an alternative during smoking cessation, it is often used as an independent substance because of its highly addictive properties (1). Smokeless tobacco products vary greatly in chemical composition. Studies have reported that most smokeless tobacco products contain carcinogenic substances such as nicotine, arsenic, cadmium, Aflatoxins, Mycotoxins, tobacco-specific nitrosamines (2-3)

Nitric oxide (NO) is the main product of cigarette smoke and has a significant negative impact on both

the environment and human health. NO is synthesized by the enzyme inducible nitric oxide synthase and hence, involved in inflammatory processes and tumorigenesis. It shows that most of the cytotoxicity attributed to NO is due to peroxynitrite produced from the diffusion-controlled reaction between NO and another free radical superoxide anion. Peroxynitrite interacts with lipids, DNA, and proteins via direct oxidative reactions or via indirect, radical-mediated mechanisms (4-5).

Nitrosative stress includes ONOO<sup>-</sup>, peroxynitrous acid (HONOO), nitrogen dioxide radical (NO<sub>2</sub>) and other types of reactive nitrogen species (RNS). Increased FNR or RNS leads to nitrosative stress (6-8). Nitrosative stress, which is the nitric oxide-mediated nitrosylation of redox-sensitive thiols, is associated with signal transduction, gene expression and the regulation of cell growth and apoptosis. RNS are known as disease-associated agents causing a number of diseases including diabetes, cancer, cardiac

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and lung disease, autoimmune disease and aging, and various infectious diseases (9-10).

NAC is a substance that increases the intracellular levels of glutathione, a major antioxidant, and modulates the oxidative, nitrosative, immune inflammatory, glutamatergic and neurotrophic pathway. NAC is a substance that can heal some physical damage caused by exposure to tobacco smoke, improve mucociliary transport and prevent oxidative damage in the lungs and other tissues. (11-13)

Amylase is an important enzyme that plays a role in the digestion of carbohydrates. In the pathological diagnosis and treatment of exocrine glands (saliva, pancreas), the measurement of serum and urine amylase levels is very important. Although habits such as ethyl alcohol, tea and caffeine etc. have been reported to affect amylase enzyme activity, the effect of chronic smoking on salivary amylase has not been fully elucidated. Furthermore, in addition to the habit of chronic smoking, it is not yet known how amylase activity is affected in those who are exposed to tobacco (nicotine) inhalation in tobacco processing units and in those who use Maras powder (14,15).

Vitamins and minerals play an important role in the regulation of catalytic activities of various enzymes and proteins. Electrolyte imbalance can lead to serious metabolic abnormalities such as coronary heart disease, kidney failure, and endocrine system disorders (16). Our aim in this study is to investigate the effects of NAC on amylase, electrolytes, vitamins and nitrosative stress in the plasma of rats treated with Maras powder, a smokeless tobacco type that contains in its structure similar chemicals to cigarettes.

## Materials and Method

In our study, upon receiving the approval of the Local Ethics Committee (2013/02), 8-10 weeks old 30 male Wistar albino rats weighing 200-350 g grown under standard conditions were obtained from the Department of Experimental Animals, Faculty of Medicine, Sutcu Imam University. Rats were kept in an environment with 50-60% humidity,  $22 \pm 1$  °C room temperature and a light pattern of 12 hours day/12 hours night and were fed with basal diet during the study.

Subjects were randomly divided into 3 groups and study groups were formed. Our study consisted of three groups. Control (n = 10), the group using Maras powder (n = 10), Maras powder+ NAC group. Nothing was given to the control group, but rats were anesthetized with ketamine in a similar way. To the Maras powder group, 200 mg smokeless tobacco

(Maras powder) was placed in the sublingual area under general anesthesia. It was waited for 15 minutes for Maras powder to be absorbed through mucosa. This procedure was repeated once a day and for 7 days. To the NAC group, 200 mg of Maras powder was given as in the Maras powder group and NAC was injected intraperitoneally with a dose of 100 mg / kg / day This procedure was also repeated once a day and for 7 days. On the 8th day, The rats were anesthetized, blood was collected from the left ventricle, and the rats were subsequently sacrificed. Blood samples were collected into ice-chilled EDTA-containing tubes. Plasma was obtained by centrifugation at  $\sim 3280$  g-force for 5 minutes at 4 °C shortly after collection and stored at  $-80$  °C until the next analysis. The levels of amylase, vitamins (A, C and E), electrolytes ( $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Cl}^-$ ) and, as the biomarkers of nitrosative stress, nitric oxide (NO) and nitrotyrosine (3-NTx) in the plasma of all three groups were measured by the method of ELISA. (Rat NO ELISA Kit; MyBioSource, Rat 3-Nitrotyrosine ELISA Kit; Rat Vitamin A ELISA Kit; MyBioSource, Cat: MBS267174, Rat Vitamin C ELISA Kit; MyBioSource, Cat: MBS721134, Rat Vitamin E ELISA Kit; MyBioSource, Cat: MBS020769, Sodium Assay Kit; Catalog: MBS2540574, General Kalium Assay Kit; Chloride Assay Kit.

**Statistical Analysis:** All results were expressed as mean  $\pm$  standard deviation. Variables distribution was assessed by the Shapiro–Wilk test. Comparisons between the groups were performed by one-way ANOVA followed by Tukey's post hoc test (the homogeneity of variances of the groups was tested using the Levene test). All data were analyzed using the Statistical Package for Social Sciences (SPSS) software (version 23; SPSS, Inc, Chicago, IL, USA). Differences were considered significant at  $p < 0.05$

## Results

When NO and 3NTx levels of groups are examined; Maras powder group had the highest NO ( $14.87 \pm 2.40$ ) and 3NTx ( $1.97 \pm 0.13$ ) levels. NO ( $10.04 \pm 1.78$ ) and 3NTx ( $1.46 \pm 0.32$ ) levels decreased in the group Maras powder + NAC.

When Vitamin A, Vitamin C and Vitamin E levels of groups are examined; Maras powder group had the lowest Vitamin A ( $0.26 \pm 0.06$ ), Vitamin C ( $9.37 \pm 0.34$ ) and Vitamin E ( $19.62 \pm 0.13$ ) levels. When amylase levels of groups are examined; Maras powder group had the highest amylase ( $177 \pm 34$ ) levels. Amylase ( $141 \pm 38$ ) level decreased in the group Maras powder + NAC.

**Table 1.** The levels of vitamins, amylase, electrolytes and nitrosative stress biomarkers in all groups

	Control	Maras powder	Maras powder+NAC	
	Mean± SD	Mean± SD	Mean± SD	p
NO (µmol/L)	9.58±1.68	14.87±2.40 <sup>ac</sup>	10.04±1.78 <sup>b</sup>	<0.05
3-NT(pg/mL)	1.25±0.25	1.97±0.13 <sup>ac</sup>	1.46±0.32 <sup>b</sup>	<0.05
Vit. A(µg/mL)	0.40±0.03	0.26±0.06 <sup>ac</sup>	0.38±0.01 <sup>b</sup>	<0.05
Vit.C (µg/mL)	12.47±0.61	9.37±0.34 <sup>ac</sup>	10.51±0.41 <sup>b</sup>	<0.05
Vit.E (µg/mL)	28.86±0.21	19.62±0.13 <sup>ac</sup>	24.74±0.16 <sup>b</sup>	<0.05
Amylase (U/L)	135±44	177±34 <sup>ac</sup>	141±38 <sup>b</sup>	<0.05
Na <sup>+</sup> ( mmol/L)	136±24	167±32 <sup>ac</sup>	140±21 <sup>b</sup>	<0.05
K <sup>+</sup> (mmol/dL)	13.0±1.6	20.6±10 <sup>ac</sup>	14.2±2.4 <sup>b</sup>	<0.05
Cl <sup>-</sup> (nmol)	297±33	361±39 <sup>ac</sup>	316±28 <sup>b</sup>	<0.05

a: Different from Control, b: Maras powder, c: Maras powder+NAC, Data are expressed as mean ± SD (n = 8). Values in the same rows with different letters (a–c) are statistically different (p < 0.05). NAC: N-acetylcysteine

When Na<sup>+</sup>, K<sup>+</sup>, Cl<sup>-</sup> levels of groups are examined; Maras powder group had the highest Na<sup>+</sup> (167±32), K<sup>+</sup> (20.6±10), Cl<sup>-</sup> (361±39) levels. Na<sup>+</sup> (140±21), K<sup>+</sup> (14.2±2.4), Cl<sup>-</sup> (316±28) levels decreased in the group Maras powder + NAC.

In a nutshell, It was detected that the levels of amylase, 3-NTx, NO, electrolyte in the group treated with NAC decreased compared to the group using only Maras powder compared to the control group and NAC group, but vitamin of levels decreased significantly, especially Vitamin A and Vitamin E of levels (p<0.05). It was pinpointed that in the group treated with NAC, the levels of amylase, 3-NTx, NO decreased compared to the group treated with Maras powder, whereas vitamin and electrolyte levels significantly increased and approached to the values of the control group (Table 1).

## Discussion

Smokeless tobacco is consumed in more than 140 countries, so it is a global problem. According to a recent survey, it causes about 0.65 million deaths annually. Consumption of these products has been reported to be associated with many diseases such as cancers, neurological disorders, oropharyngeal, esophageal, and cardiac diseases. This can be attributed to the presence of harmful chemicals together with 28 known carcinogens (17-18). Maras powder which is a type of smokeless tobacco produced from *Nicotiana rustica* L. plant (colloquially mad tobacco), is widely used in Kahramanmaraş. It has been stated that Maras powder, which is one of the smokeless tobacco varieties, has effects on the various cancer and mutation-causing effects mentioned above, especially in the formation and progression of oral, esophageal, pancreatic cancer, but also harmful effects on respiratory system,

cardiovascular system and immunological, biochemical and hematological parameters (19-20). RNS are composed of radicals and other reactive nitrogen factors that can react with other substrates. Examples of RNS are nitric oxide and peroxyxynitride. Under physiological conditions, these are balanced by a number of defense mechanisms and it should be emphasized that RNS has several physiological roles, including signaling. In extreme cases or when defenses are compromised, RNS can damage these substrates by reacting with fatty acids, proteins, and DNA. RNS produced under normal conditions is tightly regulated by balancing systems consisting of antioxidants, antioxidant enzymes and proteins. Antioxidants regulate nitrosative reactions in the body, remove radicals by scavenging RNS, reduce RNS production, thereby prevent damage caused by RNS. Nitrosative stress occurs when there is an imbalance between a relative distress in antioxidant defenses due to increased RNS production (21). NO production may cause tissue toxicity after reacting with peroxyxynitrite anions and superoxide anions producing peroxyxynitrous acid. These compounds can nitrate aromatic such as tyrosine and phenylalanine, resulting in NO-tyrosine formation (22-23). As described above, RNS can cause NO-tyrosine formation. Tyrosine nitration can be used for in vivo production of RNS (20). In one study, urine NO levels of people who use smokeless tobacco were higher than urine NO levels that did not use smokeless tobacco. In our study, NO level of the maras powder group is high (4). In NAC group, the levels of NO and 3-NTx were found to be lower than the levels of NO and 3-NTx of Maras powder group. This can be explained by the fact that NAC eliminates reactive nitrogen species by exhibiting free radical scavenging properties. Because the critical antioxidant power of NAC plays a role as a precursor to

glutathione, one of the most important antioxidants. This gives NAC the ability to scavenge radicals (24).

Vitamins A, C, E are of vital importance. These vitamins are powerful antioxidants and remove radicals such as reactive oxygen/nitrogen species from the body (25-27). In another study conducted in the USA, vitamin C levels of smokers and non-smokers were investigated. Smokers' vitamin C level was found to be lower than non-smokers (28). In our study, vitamin C levels of the group using Maras powder were found to be lower. Besides, it was found that the vitamin C levels of the Group-3 were higher than those of Group 1. We thought that N-acetyl cysteine had a scavenging property like Vitamin C, thus keeping vitamin C levels close to normal. In one study, it was found that exposure to cigarette smoke causes vitamin A reduction. In another study, smoking has been shown to increase the need for vitamin E in humans. These results support our study (29-30). Electrolytes (mainly Na<sup>+</sup>, K<sup>+</sup>, Cl<sup>-</sup>) are vital for pathological condition monitoring and clinical diagnosis. Electrolytes are necessary to maintain the balance of body fluids between membranes and to allow cells to carry electrical signals and molecules along their membranes. Cellular functions are the appropriate pH of body fluids, metabolic reactions, muscle fiber contraction, blood clotting, activity of some enzymes and components of certain proteins, enzymes and vitamins. Imbalance in electrolytes may cause detrimental effects on health due to a higher or lower electrolyte level. These effects include fatigue, muscle weakness, high risk for cardiovascular diseases, high blood pressure, renal failure, osteoporosis and respiratory problems (31-32). Studies on how smoking affects electrolytes are unclear but controversial. Mahassni et al. conducted studies to determine the effects of hookah on some electrolyte (K<sup>+</sup>, Na<sup>+</sup>, P<sup>3-</sup>, Mg<sup>2+</sup>, Ca<sup>2+</sup>, Cl<sup>-</sup>) concentrations. Mahassni et al. They found that the C values of smoker men were significantly higher than non-smokers. Although Na<sup>+</sup>, K<sup>+</sup> values were not statistically significant, smokers were found higher than non-smokers (32). In our study, the electrolyte levels of the Maras powder group increased compared to the control group. Electrolyte levels of the NAC group approached normal levels with NAC treatment. The use of smokeless tobacco is emerging as a global problem. Smokeless tobacco 'Maras powder' increases nitrosative stress. Maras powder distorts the levels of vitamins A, E, C and the balance of electrolytes in plasma. NAC has a positive function in reducing the damage caused by Maras powder.

**Limitations:** The lack of immunohistochemical and pathological findings of the oral cavity and hematological parameters limit the study.

**Ethical Issue:** The study protocol was approved by the animal ethic review committee of the Faculty of Medicine University of Kahramanmaraş Sütcü Imam.

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**Conflict of interest:** The authors declare no competing interests.

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