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# Effects of aspartame on the evaluation of electrophysiological responses in Wistar albino rats

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## Abstract

Aspartame is a non-nutritive sweetener that is used predominantly in various 'diet' and 'low-calorie' products, such as beverages, instant breakfasts, desserts, breath mints, sugar-free chewing gum, vitamins, and pharmaceuticals, consumed by millions of people who are attempting weight loss, young adults and diabetic persons. On a weight basis, the metabolism of aspartame generates approximately 50% phenylalanine, 40% aspartic acid and 10% methanol. The detailed mechanisms of the effects of aspartame on the electrophysiological response are still unclear; therefore, this study was designed to clarify whether longer-term aspartame consumption has any effect on the electrophysiological response in Wistar albino rats. The oral administration of aspartame in a safe dose of 40 mg/kg bodyweight/day (as recommended by EFSA, 2012) was tested in Wistar albino rats for a longer period (90 days). Electrophysiological responses, including heart rate variability (HRV) and electroencephalogram (EEG) pattern, were assessed in a folate-deficient animal model along with control animals using BIOPAC and EEG equipment (model RMS EEG–24 brain new-plus: RMS – Recorder and Medicare systems). In this study, the folate-deficient animal model was used to mimic human methanol metabolism in rats. After 90 days of aspartame treatment, a significant alteration was observable in the time domain [Mean RR (ms) SDNN (ms) RMSSD (ms) PNN50 (%)] and the frequency domain [LF, HF, and LF/HF ratio] with significantly impaired frequency and amplitude of the fronto-parietal and occipital EEG waves at $p \leq 0.05$. The results of this study clearly indicate that the oral consumption of aspartame reduced HRV, with sympathetic dominance and loss of vagal tone, and altered sympathovagal activity along with impairment of learning and memory, showing an additional effect on health within this study duration. The aspartame metabolites methanol and formaldehyde may be the causative factors behind the change observed.


*Keywords:* Aspartame; HRV; EEG; Learning and memory

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## 1. Introduction

Cardiovascular diseases remain the principal cause of death in both developed and developing countries, accounting for approximately 20% of all deaths worldwide annually. Aspartame (anon-nutritive sweetener) is added to a variety of foodstuffs, offering an alternative sweetening choice to diabetics, dieters, and others who must limit sugar intake. It is consumed by millions of people in numerous products such as beverages, instant breakfasts, desserts, breath mints, sugar-free chewing gum, vitamins, and pharmaceuticals [1]. Aspartame is completely metabolized in the gut and absorbed as the two amino acids aspartic acid (40%) and phenylalanine (50%) along with methanol (10%) [2]. After aspartame consumption, the concentrations of its metabolites are increased in the blood [3]. Aspartame is unstable under conditions of prolonged heating and is inappropriate for use in cooking and baking [4]. Aspartame also decomposes in liquids during prolonged storage. It is known that the rat constitutes an important model for cardio-vascular physiologic research, and ECG-based studies have long been conducted in this animal model. Cardiac activation is an electrical propagation that results in a measurable change in potential difference on the surface of the body of the subject. The resulting amplified and filtered electrical signal is the electrocardiogram and is widely used to measure the heart rate and heart rate variability [5]. The baseline variability of the heart rate (beat to beat interval) is determined on many scales; on the shortest scale, the time between each heartbeat is irregular. These short-term oscillations reflect changes in the sympathovagal balance [5]. Our hypothesis was that methanol, a metabolite of aspartame, might cause oxidative stress in the cardiovascular centres in the brain stem and/or sympathetic centres in the hypothalamus [6]. These mechanisms may affect cardiac function in experimental animals. The experimental and epidemiological data currently available to evaluate the above toxigenic risks of aspartame are insufficient and often unreliable due to the inadequate planning and conduct of previous experiments. Recently, based on previous studies, the ability of aspartame and its metabolites to alter the oxidative status of the cells via reactive oxygen species (ROS) generation and the modulation of intracellular antioxidant enzyme levels were investigated. Oral aspartame consumption at 75 mg/(kg bw/day) has been found to cause oxidative stress in the brain and liver [6,7], and oral aspartame consumption at 40 mg/(kg bw/day) has been found to cause oxidative stress in the brain [8], liver and kidney [9], and immune organs [10] and to alter immune response [11]. The inadequacy of past studies, combined with the general limited knowledge about the safety/potential toxigenic effects of substances widely present in the industrialized diet, motivated the design of this experiment.

Heart rate variability (HRV) [12] is considered to be a non-invasive measure of autonomic nervous system activity. Despite the importance of HRV as a reproducible marker of sympathetic–parasympathetic balance, the studies related to HRV in aspartame-treated animal are very few. Little is known about the effects of aspartame on electrophysiological response (HRV and EEG). The detailed mechanisms of the effects of aspartame remain unclear; the purpose of the current study is to clarify whether longer-term aspartame consumption has any effect on the electrophysiological response of Wistar albino rats.

## 2. Material and method

### 2.1. Chemicals

Pure aspartame powder and methotrexate was purchased from Sigma–Aldrich chemical, St. Louis, USA, and all other chemicals used were of analytical grade and obtained from Sisco Research Laboratory, Mumbai, India.

### 2.2. Animal model

Animal experiments were conducted after obtaining clearance from the Institutional Animal Ethical Committee (IAEC No: 01/21/2014) and the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). The animal studies were approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments. It should also be stated clearly in the text that all persons gave their informed consent prior to their inclusion in the study. The experimental animals were healthy, inbred adult male Wistar albino rats, weighing approximately 200–220 g. The animals were maintained under standard laboratory conditions and were allowed food and water ad libitum (standard rat feed pellets supplied by M/s. Hindustan Lever Ltd., India). Animals of the aspartame-treated groups were daily administered aspartame (40 mg/kg bw/day) [13,14] dissolved in normal saline orally (by means of gavage needle) for 90 days. All of the rats were housed under conditions of controlled temperature ($26 \pm 2\,^{\circ}C$) with 12-h light and 12-h dark exposure.