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Genotoxic Activity of Saccharin, Acesulfame-K, Stevia and Aspartame-Acesulfame-K in Commercial Form

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Abstract

Genotoxic activity of various concentrations of saccharin, acesulfame-K, aspartame-acesulfame-K and stevia in their commercial form was assessed. Human lymphocytes were exposed to different concentrations of saccharin, acesulfame-K, aspartame-acesulfame-K and stevia for 2 h and then subjected to alkaline comet assay system. Saccharin and the aspartame-acesulfame-K combination showed significant genotoxic activity ($P<0.0001$). Concentrations 0.5% of acesulfame-K and stevia did not induce significant genetic damage particularly stevia possesses antigenotoxic activity at 5%, 0.5% and 0.05%. Saccharin and the combination of aspartame-acesulfame-K have genotoxic activity and represent a genetic risk for consumers. Acesulfame-K and stevia are harmless and stevia even possesses antigenotoxic activity at concentrations below 5%.

Keywords: Sweeteners commercial; Genetic damage; Genotoxicity; Comet assay; Cancer; Genetic risk

Introduction

In recent years, consumers have sought to replace table sugar with less caloric and health-safe sweeteners [1,2]. Nevertheless, the knowledge about its possible genotoxic, carcinogenic and genetic effects is scarce and contradictory [3-5]. Therefore, these substances must be investigated extensively [6].

Among the most commonly used sweeteners are: saccharin (SAC), stevia (STV), acesulfame-K (AC-K) and the aspartame-acesulfame-K combination (AS-AC-K). SAC, STV and AC-K are used individually, AS and AC-K are used in combination. The World Health Organization approved an acceptable daily intake of SAC of 0-5 mg/kg [7]. Currently the use of SAC is widely debated for its genotoxicity, potential to induce cancer and its possible damage of germ cells [8-12]. Several reports [6,13-15] agree that high concentrations of saccharin induce genetic damage in lymphocytes of different mammalian species. In the year 2000 the FDA in the United States decided to remove it from the list of potential carcinogens since a study by the National Toxicology Program reported lack of carcinogenic capacity [2,16-18]. Currently, SAC is still available in the market and controversy continues over its use, without determining its genetic dangerousness.

Another sweetener STV of natural origin and relatively new has penetrated the national and international market. Several international organizations endorse its consumption [19] because apparently it has no adverse effects on human health and they employ it as a new nutritional tool, however, Nunes et al. [20] reported that STV possesses certain genotoxic capacity in liver and brain cells. There are no more reports on this regard and its clear the need to carry out more studies about its genetic dangerousness.

The World Health Organization and FAO concluded that AC-K is safe for human consumption, [21] although, several reports [6,13,22] indicate that the AC-K, in similar doses of those reported by these organizations, causes significant genetic damage and has a dose-response relation. With respect to AS, 24 reports suggest genotoxic activity, 15 of them associate it with malignant tumors in rats and in humans [23-30] and in 13, chromosomal aberrations were detected [24]. The genotoxic effects of the synergic AS-AC-K combination have not been sufficiently discussed.

To evaluate the genotoxic activity of various chemical substances and physical agents different test systems have been developed [31]. One of the most modern bioassays is the alkaline comet test reported by Singh et al. [32], which is a sensitive tool for the detection of different types of genetic damage [33,34].

Due to the polemic information about the genotoxicity of the mentioned sweeteners, their high consumption and the fact that the genetic damage may depend on the test system employed [31], in the present study, the genotoxic activity of diverse concentrations of SAC, STV, AC-K and the combination AS-AC-K was evaluated by alkaline comet assay system in human lymphocytes.

Material and Methods

Chemical substances

The commercial saccharin was obtained from NJOY Brand™, acesulfame-K from select brand™, Stevia from Svetia™ and aspartame-acesulfame-K™ from canderel products.

Obtaining blood samples

For the preparation of human lymphocytes, 6 drops of peripheral blood were obtained by annular puncture of students not older than 20 years, not exposed to environmental contaminants and medicines (information obtained through the prior application of a questionnaire in accordance with the ethical standards on human experimentation of the Declaration of Helsinki of 1975). The total blood containing the lymphocytes was centrifuged at 3,000 rpm for 10 minutes with 3 ml of

phosphate buffer (160 mM NaCl, 8 mM Na_2HPO_4, 4 mM Na_2HPO_4, 50 mM EDTA). The supernatant was removed, and the pellet was resuspended in 500 µL of phosphate buffer.

Genotoxicity evaluation

To determine the genetic damage induced by SAC, STV, AC-K and the AS-AC-K combination, the previously obtained lymphocytes were mixed with 4.5 ml of PBS, then it was divided into 5 parts of 1 ml: negative control, saccharin, stevia, acesulfame-K and the combination aspartame-acesulfame-K. The final concentration used for the first saccharin treatment was 5% and the second 0.5% for 2 h. The same concentrations and exposure time were used with the other compounds. At the end of the treatment the samples were washed 3 times with PBS and the pellet was newly suspended in 100 µl of the same PBS to be placed later in the agarose gels. The procedure was performed twice for every individual.

Alkaline comet assay

The alkaline comet test was carried out using the method of Speit and Hartmann [35] Slides were covered with agarose Normal Melting point (NMP) at 1%, leaving it to solidify and then it was removed to have a completely clean surface. Then a 0.6% Low Melting Point (LMP) agarose layer was then placed on the slide. Once solidified, another agarose layer was added (10 µl of the suspension containing the whole blood and 90 µl of the 0.5% LMP agarose), finally, a third layer of 0.5% LMP agarose was added to cover the second layer (36,37). The slides were immersed in lysis solution (2.5 mM NaCl, 10 mM Na_2EDTA, 10 mM Tris-HCl, 1% Sodium lauroyl sarcosinate, 1% Triton X-100 and 10% DMSO, pH 10) for 24 h at 11°C. Subsequently, they were placed in a horizontal electrophoresis system with electrophoresis buffer (300 mM NaOH, 1 mM Na_2EDTA) for 45 minutes. The electrophoresis was then carried out for 30 minutes at 22 V. Immediately afterwards, the slides were washed with distilled water and stained with 90 µl of ethidium bromide. The staining was carried out by immersion in distilled water for 3 minutes. Finally, the slides were rewashed with distilled water for 10 minutes.

Observation and counting of comets

Fluorescence microscope with an excitation filter 515-560 nm was used for the count of comets. The Tail Length was measured with the Comet assay system II software.

Statistical analysis

The statistical software Statplus 2 was used to perform the analysis of variance (ANOVA) and the tests of Dunnett and Fisher. A confidence level of 0.05 was used.

Results

Figure 1 shows the evaluation of the genotoxic activity of various concentrations of SAC, STV, AC-K and the combination AS-AC-K. Significant genetic damage (P<0.0001) was observed in the 5% and 0.5% concentrations of SAC and the combination AS+AC-K. SAC induced the greatest genetic damage and a dose-response relation was observed. A similar behavior, but of lesser degree was observed for the combination AS-AC-K.

The concentrations 0.5% of STV and AC-K did not present significant difference (P<0.0001) with respect to the negative control, although, the concentrations 5% did (P<0.0001), but with a clear decrease in basal genetic damage.

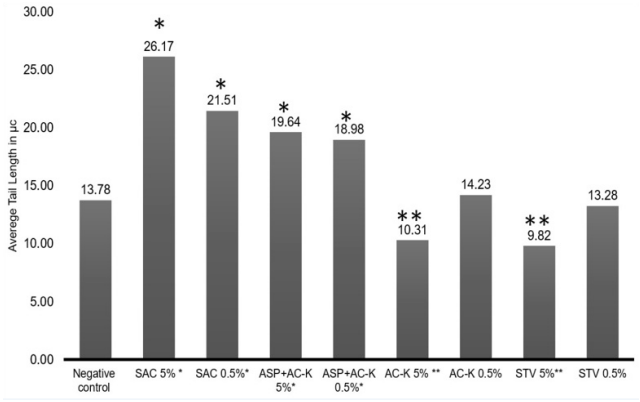

Figure 1: Average migration of DNA (tail length) in human lymphocytes exposed to different concentrations of saccharin, the combination aspartame-acesulfame-k, acesulfame-k and stevia. *Significant difference with respect to the negative control (P<0.0001), **Significant difference (P<0.0001) with decrease in migration average.

Figure 2 shows the genotoxic activity induced by different concentrations of STV. Only the concentration of 10% showed significant genotoxic activity with respect to the negative control. The lower concentrations showed significant difference with respect to the negative control but with values below the negative control, indicating decreased basal genetic damage.

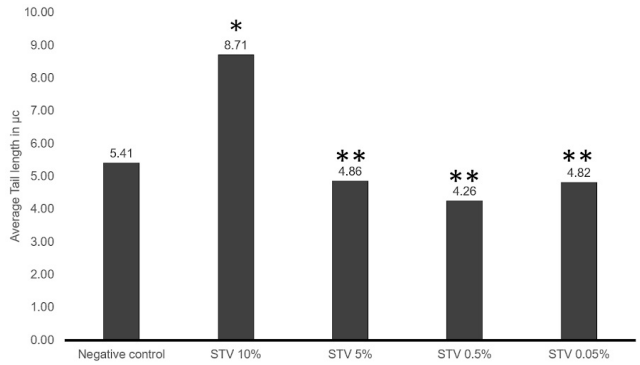

Figure 2: Average migration of DNA (tail length) in human lymphocytes exposed to different stevia concentrations. *Significant difference with respect to the negative control (P<0.0001), **Significant difference (P<0.0001) with decrease in migration average.

Discussion

SAC, ASP, AC-K and STV are the most commonly used sweeteners and constitute an alternative to treat or control various diseases [19,38,39], however, there is controversy about their genetic dangerousness [6,40,41], therefore, it is essential to increase the

knowledge about its genotoxic activity, particularly in its commercial presentation.

Our data about the genotoxicity of commercial SAC showed strong genotoxic activity and an apparent dose-response relation. These data complement previous studies with chemically pure saccharin [6,13-15] also reporting high genotoxic activity, which suggests the genetic dangerousness of SAC in all its presentations. SAC was also linked to the appearance of cancer and was taken off the market due to its genetic dangerousness [1], it was later put back on the market due to lack of scientific evidence [16-18]. Given this ambiguity of data, the need to determine conclusively the genetic dangerousness of SAC is clear. The genotoxicity of SAC in its commercial form here demonstrated, evidences the risk to which the population is exposed: increase in cases of cancer and hereditary diseases. Additionally, it is advisable to carry out more studies about its carcinogenic capacity, which is highly polemic [8-12].

AC-K has been designated as safe for the population by the World Health Organization, but it is also associated with genotoxic activity [13,22]. Our results show that the 0.5% concentration of AC-K did not generate genetic damage, which differs from other reports where AC-K was used in pure form [6,13,22]. The 5% concentration didn't show genotoxic effect either, however, it presented significant difference from the negative control with a reduction of the average migration (Tail length) even below the average of the negative control (Figure 1). We conclude that AC-K has no genotoxic activity at relatively low concentrations. The differences in the genotoxicity of AC-K could be explained by the different test systems used [32] and the use of relatively diluted AC-K from commercial forms. ASP is not commonly used individually; it is combined with different sweeteners to generate a synergic sweetening effect [42]. The chemically pure form of ASP has been directly related to genotoxic activity and the ability to cause cancer, [24-30,42] although, Mukhopatahy et al. [24] reported an absence of genotoxicity. Our data indicate that the ASP-AC-K combination possesses genotoxic activity in its commercial form with an apparent dose-response relation. Since our results with AC-K show absence of genotoxic activity and we observed that the combination AC-K-ASP is genotoxic, we deduce that the genotoxicity of this combination is due to ASP, which indicates its genetic dangerousness. Because of our data we suggest not consuming this combination. Nonetheless, it is necessary to conduct further studies in different test systems of these combinations and the chemically pure form of the ASP to test the genetic damage to the consumer in long term.

STV results indicate absence of genotoxicity in concentration 0.5% (Figure 1). These data are consistent with previous reports [43,44]. The 5% concentration not only showed absence of genotoxicity but antigenotoxic capacity, data that contrast with those obtained by Nunes et al. [21] who reported STV genotoxic activity.

The evaluation of the genotoxic activity of STV in wider ranges of concentration (Figure 2) (10%, 5%, 0.5% and 0.05%) showed an interesting behavior: the concentration of 10% (equivalent to 10 g in 100 mL) showed great genotoxic activity that begins to decrease in the rest of the concentrations reaching even, antigenotoxic levels. This behavior may explain the absence of genotoxicity found by Sekihashi et al. [43] or the genotoxicity reported by Nunes et al. [21]. Similar behavior has not been previously reported and indicates genetic dangerousness of STV only in very high concentrations and it's even beneficial and harmless in concentrations lower than 5%.

The comet test turned out to be an excellent to Singh et al. [33] for the evaluation of the genotoxic activity, particularly of SAC, STV, AC-K and the combination AS+AC-K in its commercial form, nevertheless, since the detection of genetic damage may depend on the test system employed [32] it is necessary to carry out more studies with different test systems.

It was clear that SAC and AS+AC-K are genotoxic, especially SAC. We can infer that both compounds in all their presentations will have this result, therefore, they aren't suitable for human consumption. STV and AC-K showed to be innocuous and even STV acted like a powerful antigenotoxic substance in concentrations of 0.05% of the commercial form. Therefore, the need to conduct more research in this regard is a necessity.

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