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Original article

Demonstrating adverse effects of a common food additive (sodium sulfite) on biochemical, cytological and histopathological parameters in tissues of albino Wister rats

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Abstract

Introduction: Sulfites have been used as preservatives in food and drugs. The use of sulfite-containing compounds on fruits and vegetables was banned because of potential allergic reactions, and those suffering from asthma are at elevated risk of sulfite sensitivity.
Method: The present study investigated the effect of daily administration of sodium sulfite (Na_2SO_3) on female albino rats at doses of 200, 500, and 1000 ppm for 12 weeks. Impacts on growth, blood picture, genotoxic effect, some biochemical parameters and histopathological characteristics were examined.
Results: The results revealed that Na_2SO_3 causes a significant decrease in body weight, red blood cells (RBC) count, hemoglobin (Hb) concentration, hematocrit (HCT) value, white blood cells (WBC), and glucose level, while there was a significant increase in serum activity of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) as well as the serum urea and creatinine levels in the treated rats. Cytogenetic analysis showed various types of chromosomal aberration such as fragments, chromosome ring, chromatid break, stickiness of chromosome, end to end association, translocation, and centric fusion. Histopathological examination of the experimental animals indicated little sinusoidal dilatation in rats treated with 200 ppm of Na_2SO_3. Hepatic vacuolation, large sinusoidal dilatation, degenerative changes and cellular congestion were shown in liver of rats treated with 500 and 1000 ppm of Na_2SO_3 when compared to the control group.
Conclusion: Administration of Na_2SO_3 to rats exhibited serious effects on both liver and kidney cells.

Keywords: Safety assessment; Sulfur-containing compounds; Toxicity; Sodium sulfite; Kunafa powder; Chromosomal aberrations

Introduction

Sulfite is one of the most ubiquitous food additives, and is widely used as a blanching and preservative agent in different foodstuffs, to improve the appearance of food and prevent bacterial growth [1,2]. Sulfites are multipurpose compounds found as calcium, potassium, or sodium salts in food and pharmaceutical industries as preservatives or antioxidants. They are also used to slow food oxidation and can sometimes affect the smell and taste of foods [3,4]. Five sulfite salts including sodium metabisulfite ($Na_2S_2O_5$), potassium metabisulfite ($K_2S_2O_5$), sodium bisulfite ($NaHSO_3$), potassium sulfite (K_2SO_3), and sodium sulfite (Na_2SO_3) are commonly used as antioxidants in food and pharmaceutical preparations [5,6]. Generally recognized

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as safe (GRAS) compounds, when used at the minimum amount required for the intended purpose within foods and cosmetics, sulfite-containing compounds are considered safe by the US Food and Drug Administration (FDA). Sulfite-containing compounds including sodium sulfite (GRAS 182.3798), sodium bisulfite (GRAS 182.3739), and sodium metabisulfite (GRAS 182.3766) are on the FDA's GRAS list [4].

Allergic reactions are occurring to sulfite-containing compounds in foods. About 1% of people in the United States are sulfite hypersensitive according to FDA estimation. Exposure to sulfite-containing compounds induced adverse clinical effects in sensitive individuals, ranging from dermatitis, urticaria, hypotension and diarrhea to life-threatening anaphylactic and asthmatic reactions. While contact sensitivity to sulphite additives in topical medications was being recognized, skin reactions also occur after ingestion of or parenteral exposure to sulphites. Sulphites may also contribute to chronic skin and respiratory symptoms [7,8].

Owing to the potential toxicity, the sulfite in foods is strictly limited in many countries [2]. The FDA requires labeling when sulfites are used as preservatives in a final product; however, when sulfites are used in food-processing steps, labeling is required only when the sulfite residual is more than 10 ppm [4,9]. Once ingested, sulfite salts react with water leading to generation of bisulfite (HSO_3^-), sulfite (SO_3^{2-}) and sulfurdioxide (SO_2). It was shown that ingested sulfite enters the systemic circulation by gastrointestinal absorption and distributed essentially to all body tissues including the brain [5]. Sulfite can react with a variety of humoral and cellular components and can cause toxicity [6]. The effect of Na_2SO_3 (E221) was investigated in human peripheral blood mononuclear cells (PBMC), wherein sodium sulfite demonstrated a suppressive influence on the activated Th1-type immune response [10]. Sulfite is detoxified by sulfite oxidase in the mammalian tissues. Sulfite oxidase, a molybdenum-containing enzyme located in the intermembranous space of the mitochondria, oxidizes sulfite to sulfate in a two-electron oxidation step and protects cells from sulfite toxicity [11,12]. If there is deficiency of sulfite oxidase or exposure to excessive sulfite, the sulfite undergoes one electron oxidation reactions, catalyzed by peroxidases to form sulfur trioxide anion radical (SO_3^-). The sulfite radicals can react with oxygen molecules forming sulfite peroxyl radical ($SO_3OO^{\cdot}$) and sulfate radical (SO_4^-) [6,13,14]. Reactive oxygen species (ROS) are produced by a number of cellular oxidative metabolic processes, monoamine oxidases, mitochondrial respiratory chain and PLA2 pathway [6,15].

Considerable amounts of exogenous sulfites are consumed *via* food, beverages and pharmaceutical products. The concentration of sulfites in dried fruits, wine as well as lemon and lime juice, is estimated to be 1.6 mM sulfur dioxide equivalents [16]. Meng and Zhang [17] reported that the SO_2 derivatives (bisulfite and sulfite) may induce chromosomal aberrations (CA), sister chromatid exchanges (SCE) and micronuclei (MN) in cultured human blood lymphocytes *in vitro*. Such results suggest that SO_2 and its derivatives are clastogenic and genotoxic agents.

In some countries, sodium sulfite (Na_2SO_3) is commercially known as *Kunafa* powder and used primarily as a dough conditioner during manufacturing of an Arabic dessert *Kunafa*. Although Na_2SO_3 is still used as additive in many foods, there is limited existing information concerning the biochemical changes associated with toxicity of Na_2SO_3. This poses a great threat to food safety and public health especially to who consume *Kunafa* products with no information about its adverse effects on body tissues. The present study aimed to demonstrate the biochemical, cytological and histopathologic changes in serum, liver and kidney tissues of rats following exposure to different doses of Na_2SO_3.

Methods

Sodium sulfite Na_2SO_3 (96% minimum purity, crystal, industrial grade, production date: May, 2013, expiry date: May, 2016) was obtained from the local market in Fayoum City (Egypt).

Animals and dosing

Healthy female albino Wister rats with average body weight of 110 ± 3 g were obtained from Faculty of Veterinary Medicine, Cairo University (Egypt). Ethical approval was obtained from ethical committee of the Fayoum University (Permission number 6200, 2011). The work has been carried out in accordance with EU Directive 2010/63/EU for animal experiments (http://ec.europa.eu/environment/chemicals/lab_animals/legislation_en.htm). Animals were randomly selected and equally divided into four groups (five per group), approximately equal in weight. The animals were housed in cages and subjected to 1 week acclimatization before starting of experiment at atmosphere of 12 h dark/light cycle, at $25 \pm 2\,^{\circ}C$ and provided with normal diet (standard laboratory chow). Group 1 served as control, while groups 2, 3 and 4 were given daily oral doses of Na_2SO_3 dissolved in drinking water at concentrations of 200, 500, and 1000 ppm, respectively for 12 weeks.

At the end of the experiment, blood was collected from the retro-orbital sinus under ethyl ether anesthesia after overnight fast. Blood samples were centrifuged at 4000 rpm for 15 min at $4\,^{\circ}C$ to recover serum. The anticoagulant blood samples were used for determining hematological parameters. Meanwhile, the serum was used for blood analysis.

Body weight and relative organ weight of rats

Animals were weighed at the beginning and at the end of the experimental period. Three animals of each group were killed by decapitation. The liver and kidney were carefully dissected out and weighed. The relative organ weight of each animal was calculated as follows:

$$\text{Relative organ weight} = \frac{\text{Absolute organ weight(g)}}{\text{Body weight of rat on sacrifice day(g)}} \times 100$$