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Comparative haemato-immunotoxic impacts of long-term exposure to tartrazine and chlorophyll in rats

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ABSTRACT

The haemato-immunotoxic effects of the food colourants tartrazine and chlorophyll were evaluated. Thirty adult Sprague Dawley rats were distributed into three groups and orally administered water, tartrazine (1.35 mg/kg), or chlorophyll (1.35 mg/kg) daily for 90 days. Erythrogram and leukogram profiles were evaluated. The lysozyme, nitric oxide, phagocytic activity, and immunoglobulin levels were measured. Histological and immunohistochemical evaluations of splenic tissues were conducted. Changes in the interleukin (IL) 1β, 6, and 10 mRNA expression levels were assessed. In the tartrazine-treated rats, a significant anaemic condition and marked leukocytosis were observed. Both the innate and humoural parameters were significantly depressed. Different pathological lesions were observed, including red pulp haemorrhages, vacuolation of some splenic cells, focal hyperplasia of the white pulp, and capsular and parenchymal fibrosis. A marked increase in vascular endothelial growth factor (VEGF) and endothelial nitric oxide synthase (e-NOS) immunolabelling was evident. Marked upregulation of IL-1β, IL-6, and IL-10 was recorded. In contrast, the chlorophyll-treated rats showed minimal haemato-immune responses. These results indicate that tartrazine exerts haematotoxic and immunotoxic effects following long-term exposure, whereas chlorophyll is a less hazardous food colourant.

1. Introduction

Colour is one of the chief features of food [1]. Food colourants, which are a functional class of food additives, are used in the food industry to make food attractive to consumers and to add variety [2]. Throughout the past 50 year, the quantity of synthetic dye used in foods has increased by 500% [3]. A recent report on products and food colourant data from 810 brands in USA reported that the most common synthetic food colourant was tartrazine, which was present in 20.5% of the products marketed for consumption [4].

Tartrazine (E102, FD&C Yellow No. 5, C.I. No. 19140) is an azo dye and salt of 3-carboxy-5-hydroxy-1-(*p*-sulfophenyl)-4-(sulfophenylazo) pyrazolone [5]. Tartrazine is the most commonly used food colourant to achieve yellow shades in sweets, jellies, juices, jams, mustard, and sodas. Additionally, it has been extensively used to colour human pharmaceuticals, such as vitamin capsules, antacids, cosmetics and hair products [6,7]. Furthermore, in many developing countries, this food colourant has been adopted as a substitute for saffron in cooking [8]. The acceptable daily intake (ADI) for humans is 7.5 mg/kg body weight [9].

Toxicokinetic studies showed that only 2% of the ingested tartrazine is directly absorbed and most tartrazine is broken down into smaller metabolites like sulfanilic acid and aminopyrazolone in the colon [10]. The former metabolites can be absorbed to a better extent than tartrazine and could be distributed to different body system inducing adverse reactions [11].

Tartrazine is reportedly one of the most controversial colouring agents. Many studies have demonstrated that this food dye is safe for consumption at the ADI level because no dangerous effects have been recorded in either humans or experimental models [12,13]. However, other studies have reported that tartrazine can exacerbate asthma, angioedema, and urticaria in atopic patients [14,15]. Tartrazine was also

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found to induce neurotoxic [16], reprotoxic [17], genotoxic and mutagenic effects [18,19]. Furthermore, a dose-response effect was observed in the incidence of behavioural changes in children (i.e., restlessness, irritability, and sleep disturbance) after tartrazine ingestion [20].

Chlorophylls (E 140) are authorized as food additives in the European Union (EU) in accordance with Annex II to Regulation (EC) No. 1333/2008. These additives are used as food colouring agents in cakes, beverages, sweets, and ice cream [21]. The few available toxicological have not specifically investigated chlorophylls as food additives but instead have evaluated chlorophyll intake in the regular diet [22]. The US Food and Drug Administration (FDA) allocated an ADI of 7.5 mg/kg per day for chlorophyll [23].

Henderson and Long [24] orally dosed chlorophyll to rats and found uncharacterized derivatives dispersed in the liver, lymph nodes, and spleen. More recent in vitro investigation of the digestive behaviour of chlorophyll has reported pheophytins and pheophytin epimers formation as the primary small intestinal derivatives [25].

Some studies have reported that chlorophyll can induce indistinct genotoxic and carcinogenic effects [26,27]. In a 13-week sub-chronic oral toxicity study by Furukawa et al. [28], F344 rats of both sexes were fed a diet containing 0, 0.18, 0.55, 1.66 or 5% chlorophyll. No mortalities were recorded during the administration period, and no changes in body weight or food intake were recorded in any of the treatment groups. Some haematological, serum biochemical and histopathological changes were recorded for the 5% treatment group, but these changes did not pose any apparent toxicity. These findings showed that exposure to 1.66% chlorophyll in the diet for 13 weeks did not cause any changes in rats and that 5% chlorophyll feeding was not evidently toxic.

Little information is available on the effects of tartrazine and chlorophyll on innate and humoural functions. The present study was conducted to assess the effect of 90-day oral exposure to 10 times the ADI of tartrazine and chlorophyll on haematological assessments (erythrogram and leukogram), innate immune indicators (lysozyme activity, phagocytosis, and nitric oxide [NO] level), and humoural immunoglobulin levels. Histopathological and immunohistochemical imaging of the spleen was also performed. Furthermore, the induction of interleukin (IL) 1β, 6, and 10 mRNA expression was examined by real-time polymerase chain reaction (PCR) in the rat spleen.

2. Materials and methods

2.1. Test compounds and other reagents/chemicals

Tartrazine (E102, CAS 1934-21-0, purity 86.7%) and chlorophyll (E 140, 479-61-8, purity 95%) were purchased from Sigma (St. Louis, MO). Each compound was diluted to a working stock concentration using distilled water. Commercial enzyme-linked immunosorbent assay (ELISA) kits (Kamiya Biomedical, Tukwila, WA) were obtained to determine the serum immunoglobulin G (IgG) and IgM concentrations and the lysozyme activity level (MyBioSource, San Diego, CA). All other reagents/chemicals were purchased from Sigma (St. Louis, MO) and were of analytical grade.

2.2. Animal grouping and experimental design

Thirty adult male Sprague Dawley albino rats were purchased from the breeding unit of the National Research Centre (Giza, Egypt). All rats were housed in steel mesh cages maintained at a controlled temperature (21–24 °C) with a relative humidity of 50–60% and a 12-hour light–dark cycle. All rats had ad libitum access to standard rodent chow and tap water throughout the study. The rats were acclimated 2 weeks prior to use in any study described herein.

At the start of the experiment, the rats were weighed and randomly divided into three groups containing 10 rats each.

Group I (C): The control rats received distilled water.
Group II (tartrazine): The rats in this group were administered 75 mg/kg bwt of tartrazine calculated for humans and modified according to Paget and Barnes [29] to 1.35 mg/kg bwt.
Group III (chlorophyll): The rats in this group were administered 75 mg/kg bwt of chlorophyll calculated for humans and modified according to Paget and Barnes [29] to 1.35 mg/kg bwt.

The concentrations used were equal to 10 times the ADI. For 90 consecutive days, the rats were given all treatments at a dose of 1 ml/100 g bwt once daily through a 20-G feeding needle.

The rats were observed during the experimental period for signs of toxicity, morbidity, and mortality. All rats were weighed once per week, and the dose volumes were adjusted accordingly.

The experimental procedures were performed in line with the general guidelines of the National Institutes of Health for the Care and Use of Laboratory Animals in scientific investigations and were approved through the Ethics of Animal Use in Research Committee of Cairo University, Egypt.

2.3. Sampling

At the end of the dosing period (90 days), the rats were fasted overnight after exposure. Three separate blood samples were collected from the retro-orbital plexus from each rat:

1. The first blood sample was collected into an EDTA tube for use in haematological evaluations.
2. The second blood sample was collected into a tube without EDTA and allowed to coagulate for 20 min at room temperature; then, the sample was centrifuged at 3000 rpm for 10 min. The generated serum was isolated and stored at −20 °C prior to use (within 2 weeks) in the biochemical assays outlined below.
3. The third blood sample was collected into a lithium heparinized tube to separate neutrophils and macrophages for measurement of phagocytic activity and the nitric oxide level, respectively.

Then, all rats in each group were weighed and euthanized by cervical dislocation. The spleen was removed, washed with saline and weighed. Small sections of the spleen were collected and fixed in 10% buffered neutral formalin solution for histopathological and immunohistochemical examinations. The remainder was directly preserved in a liquid nitrogen container and stored at −80 °C for real-time PCR analysis.

2.4. Haematological evaluation

Determinations of the total red blood cells (RBCs), packed cell volume (PCV), haemoglobin (Hb), mean cell volume (MCV), mean corpuscular haemoglobin concentration (MCHC), total leukocytes, lymphocytes, granulocytes, and monocytes were conducted according to the method of Feldman et al. [30] using a HemaScreen 18 automated haematology analyser (Hospitex Diagnostics, Sesto Fiorentino, Italy). Total leukocyte counts were performed using an automated analyser, and the differential leukocyte counts were conducted via the manual method of Dacie JV [31].

2.5. Nitroblue tetrazolium assay (NBT) measurement of polymorphonuclear granulocyte (PMN) phagocytic activity

2.5.1. Neutrophil isolation

Neutrophils were isolated using the protocol of Oh et al. [32] by layering whole blood over the density gradient medium, centrifuging, collecting the neutrophil layer, and lysis of the residual erythrocytes by hypotonic lysis. The cells were washed, counted, and resuspended in buffer to the proper concentration. The cell viability of each sample was

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