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Comparative protective effects of royal jelly and cod liver oil against neurotoxic impact of tartrazine on male rat pups brain

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ABSTRACT

This study is aimed to evaluate the possible neurotoxic effect of tartrazine (T), an extensively used synthetic azo dye, as well as to determine the potential modulatory role of cod liver oil (CLO) or royal jelly (RJ) against such effects. For this purpose, thirty-six male rat pups were allocated into six groups. The 1st group received distilled water (control group), the 2nd group was given 300 mg RJ/kg bw (RJ group), the 3rd group was given 0.4 ml CLO/kg bw (CLO group), the 4th was given 500 mg T/kg bw (T group). The 5th group was given T concurrently with RJ (TRJ group) and the 6th group was given T concurrently with CLO (TCLO group), at the same doses as the former groups. All treatments were given orally for 30 consecutive days. The concentrations of different brain neurotransmitters, gamma amino butyric acid (GABA), dopamine (DA) and serotonin (5HT) as well as the antioxidant and oxidative stress biomarkers were measured in the brain homogenates. An immunohistochemical staining of the cerebral cortex was applied with the anti-ssDNA antibody (an apoptotic cell marker) to reveal the changes in brain structure. The T group revealed a significant decrease in the concentration of the brain neurotransmitters, a sharp shortage in the level of antioxidant biomarkers (super oxide dismutase, catalase and the reduced glutathione), a marked increase in malondialdehyde levels, and numerous apoptotic cells in the brain cortex compared with the other groups. Interestingly, all the previously mentioned parameters were almost retrieved in both the TRJ and TCLO groups compared to the T group. These results conclusively demonstrate that RJ and CLO administration provides sufficient protection against the ruinous effects of T on rat pups brain tissue function and structure.

1. Introduction

Color is one of the main characteristics of food. When the natural color of food is lost during processing, synthetic colors can be added to enhance the attractiveness and zest of food. Tartrazine (T) (E 102, FD and C Yellow) is an orange-colored powder extensively used to color food products, known as synthetic lemon yellow. It is an artificial azo dye derived from coal tar (Elhakim and Heraud, 2007). T has been widely used to color human pharmaceuticals such as capsules of vitamins, antacids, cosmetics and other hair products. It has been also added to give a pleasant color to cake mixes, jams, jelly, flavored chips, chewing gums, biscuits, sauces and ice cream (Babu and Shenolikar, 1995; Reyes et al., 1996; Walton et al., 1999). Furthermore, T has been used as a substitute for saffron for cooking in many developing countries (Mehedi et al., 2009). The acceptable daily intake (ADI) for T is 7.5 mg/kg bw (Toledo, 1996; Hirschbruch and Torres, 1998; Walton et al., 1999).

The azo compounds, with the (N=N) functional group and aromatic rings linked to them, are reductively cleaved into aromatic amines. Some of these amines are toxic, carcinogenic and mutagenic (Chung, 2000; Zhang and Ma, 2013).

Several investigations revealed that T was responsible for several reactions including allergic reactions and hyperactivity, especially in children. Gautam et al. (2010) and Tanaka (2006) found out that T administered to mice in the diet had effects that lead to hepato-cellular damage, and biochemical and reproductive

Abbreviations: T, tartrazine; RJ, royal jelly; CLO, cod liver oil; TRJ, tartrazine and royal jelly co-treatment; TCLO, tartrazine and cod liver oil co-treatment; HE, hematoxylin and eosin; GABA, gamma amino butyric acid; DA, dopamine; 5HT, serotonin; SOD, super oxide dismutase; CAT, catalase; GSH, reduced glutathione; MDA, malondialdehyde; DHA, docosahexaenoic acid.

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alterations in high doses, and even in low doses. Mpountoukas et al. (2010) indicated that T could potentially be genotoxic for human lymphocytes and could bind directly to DNA. T also had a dose dependent manner DNA-damaging effect estimated by comet assay in fresh blood collected via the abdominal aorta of Wister rats (Imane et al., 2012). Gao et al. (2011) also indicated that T could cause defects in learning and memory in mice and rats.

Thousands of new chemicals are introduced every year. Unfortunately, the Environmental Protection Agency reported that about 25% of these chemicals had neurotoxic effects especially for the developing brain. Thus in recent years, there has been a great interest in naturally obtained formulas that mitigate this effect. One of the most beneficial and remedies for such effects, used also as a dietary supplement, is royal jelly (RJ) (Silici et al., 2010).

Royal jelly (RJ) is secreted by the cephalic glands of nurse bees (*Apis mellifera*). It is a whitish to yellow viscous substance and varies in viscosity according to water content and age (Takenaka et al., 1986). Interestingly, RJ has an amazing collection of beneficial dietary components such as free amino acids, sugars, proteins, vitamins, fatty acids and minerals (Sabatini et al., 2009). Furthermore, it has a wide range of pharmacological uses including as a potent antioxidant (Nakajima et al., 2009), immunostimulant (Okamoto et al., 2003), and hepatoprotective agent (Kanbur et al., 2009). Also, it has a neurotrophic effect through different mechanisms on both developing and mature CNS (Krylov and Sokolskii, 2000). Over and above, RJ possesses antitumor, antibacterial, hypoglycemic, anti-inflammatory and anti-hypercholesterolemic properties (Kamakura et al., 2006; Nagai et al., 2006).

Cod liver oil (CLO) is another natural remedy that has many proven benefits. CLO, a rich source of omega-3 fatty acids, is used as omega-3 fatty acids supplementation (Perveen et al., 2013). Several years ago, many researchers elucidated the beneficial role of CLO in maintaining the health of bones in adults, and the prevention of rickets in infants. There are many other immense health benefits of this natural formula including anti-inflammatory properties caused by the omega-3 fraction (Kehn and Fernandes, 2001). It has antidiabetic (Hunker et al., 2002) and neuro-protective qualities in epileptic conditions caused by damage in the hippocampus (Ferrari et al., 2008). Also, CLO reduces cardio-metabolic risk factors (Abeywardena and Patten, 2011) and ameliorates cognitive impairment induced by chronic stress (Trofimiuk and Braszko, 2011). Moreover, it has anticancer (Dyck et al., 2011), hepatoprotective (Salama et al., 2013), antidepressant and anti-anxiety effects (Perveen et al., 2013).

Neurotransmitters play unique trophic roles in the development of the brain. Accordingly, many drugs and environmental toxicants that promote or interfere with neurotransmitter production levels induce neurodevelopmental abnormalities by disrupting the intensity of neurotrophic activity (Slotkin, 2004). Among these neurotransmitters are gamma amino butyric acid (GABA), dopamine (DA) and serotonin (5HT) which are considered to be the most important neurotransmitters that reflect brain function. GABA is an inhibitory neurotransmitter which slows down the actions of the neurons, so as to avoid them from getting overexcited. The role of DA appears to control the voluntary movements of the body (El-Ansary et al., 2011). 5HT is considered to have a profound effect on mood, emotion, and anxiety.

There was little available literature concerning the neurotoxic effect of T in previously carried out investigations. Therefore, the present study was designed to highlight the changes in the function and structure of the brain following oral administration of tartrazine in rat pups and the degree of protection achieved by concurrent administrations of either RJ or CLO with T.

2. Materials and methods

2.1. Chemicals

Tartrazine $C_{16}H_9N_4O_9S_2Na_3$ (T), cod liver oil (CLO) and royal jelly (RJ) were orally administered to different experimental rat groups for one month. The T (food yellow No. 19 140) was obtained from Loba Chemie Pvt. – India. The purity of the chemical was more than 90.01% (MW. 534.4). The CLO was obtained from Seven Seas Ltd., Hedon Road, Hull, England, pure cod liver oil 150 ml. The RJ was obtained from the Plant Protection Department, Faculty of Agriculture, Zagazig University. It was divided into equal amounts of 300 mg each and stored at −20 °C until used.

2.2. Experimental animals and treatments

Thirty-six male Sprague–Dawley rat pups of locally bred strain (45–55 g) were obtained from animal house, Faculty of Veterinary Medicine, Zagazig University. The pups were kept in stainless steel cages in a clean room with a controlled temperature (20–25 °C) free from any chemical contamination in a 12 h dark/light cycle. The animals were fed a standard diet beside water ad libitum. The management of the animals and the experimental protocols were conducted as stipulated in the Guide for Care and Use of Laboratory Animals Guidelines of the National Institutes of Health (NIH), and approved by the local authorities of Zagazig University, Egypt.

The pups were allocated into six equal groups (6 animals/group) and given a daily oral dose of different treatments by gavage method for 30 consecutive days. Group I (control group) was administered 0.4 ml distilled water. Group II (RJ group) was administered RJ (300 mg/kg bw) (Karadeniz et al., 2011). In group III (CLO group), the pups were administered CLO at a dose of (0.4 ml/kg bw) (Fathia et al., 2011). In group IV (T group), the pups were given T 500 mg/kg bw (based on previous reports by Amin et al., 2010; Gao et al., 2011). In group V (TRJ group), the pups were co-adminstered RJ with T, and in group VI they were co-administered CLO with T, at the same previously mentioned doses and duration.

2.3. Tissue preparation for microscopic investigation and homogenate

At the end of the experiment, the rats were euthanized under diethyl ether anesthesia and the right and left cerebral cortices were removed. The right cerebral cortices from all groups were fixed with 4% paraformaldehyde. After overnight fixation, specimens were washed in distilled water, dehydrated in graded alcohol and embedded in paraffin. Subsequently, 3-μm-thick paraffin sections were deparaffinized, and rehydrated. Some were stained with hematoxylin and eosin (HE) and observed by light microscopy for histopathological examination according to Bancroft and Stevens (1996). The other sections were kept for immunohistochemical staining. The left cerebral cortices were cut into small pieces for homogenate preparation. The homogenate was prepared using an electrical homogenizer by mixing 0.5 g of tissue with 5 ml phosphate buffer saline at 4 °C. Then homogenates were centrifuged at 3000 rpm for 15 min. Then, the collected supernatant was conserved (at −20 °C) until further use for the biochemical examination (neurotransmitters and oxidative stress biomarkers assays).

2.4. Assay of neurotransmitters

The concentrations of different brain neurotransmitters (gamma amino butyric acid (GABA), dopamine (DA) and serotonin (5HT)) were measured in the brain homogenates. GABA concentration was measured using a specific rat ELISA immunoassay