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The impact of tartrazine and thymoquinone on rats' lungs

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Abstract

Aim: The study aimed to investigate the changes induced by Tartrazine (T) and Thymoquinone (TQ) in rat lung tissues.

Materials and Methods: Thirty-two rats were divided into four groups, 8 animals in each group: Control, T, TQ and T. The experiments were conducted for 21 days. Oxidant-antioxidant parameters were determined in rat lung tissues.

Results: An increase was observed in Malondialdehyde (MDA) parameters in the lung tissues of rats in the T-treatment group when compared to all other groups, a significant decrease was observed in reduced glutathione (GSH), superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), and catalase (CAT) levels. It was observed that TQ administration led to a significant increase in antioxidant capacity when compared to other groups. Coadministration of T and TQ led to improvements in oxidant and antioxidant parameters when compared to the T group.

Conclusion: In this first study on the correlation between T and TQ, T administration led to damages in lung tissues. It induced oxidative stress via the increase in oxidant capacity. TQ led to an increase in antioxidant capacity. We recommend TQ consumption to maintain strong antioxidant capacity against oxidative stress damage and T toxicity.

Introduction

Food additives are defined by the European Union Directive EC 1333/2008 as substances that are not consumed as food products but added to food products to flavor, color, preserve these items, or to adjust acidity [1]. Artificial colorants (azo (N=N) dyes), the largest group of food additives, have been used in the food industry for several years to change or disguise the appearance, color and texture of food products. Artificial colorants are preferred over natural dyes due to their resistance to pH changes and weather, and low cost. These intended uses could be observed in beverages, confectionery, and bakery products [2,3]. Tartrazine (T) is generally yellow or orange. T is a synthetic azo dye. T was designated as E102 by the European Food Safety Authority (EFSA). It is called FD&C Yellow 5 T by the US Food and Drug Administration (FDA). The chemical structure of T is as follows: $C_{16}H_9N_4Na_3O_9S_2$. T is used to change the appearance and color of foodstuffs to obtain more appetizing and attractive food products [4, 5]. It is commonly used in potato chips, ice creams, jellies, jams, canned foods, chewing gums, and in pastry. It is widely used due to its low cost, reflecting its preference in several developing countries [6].

Several studies reported allergenic, clastogenic, mutagenic, and carcinogenic effects of T. Thus, both long and short-term biochemical, mutagenic, and acute toxicity studies were conducted for consumer safety [7]. It was reported that T led to hepatotoxicity, nephrotoxicity, neurotoxicity, asthma, anemia, leukopenia, skin rashes, behavioral disorders, and decreased enzyme functions in mice [8-15].

Black cumin, Nigella sativa (NS) species is a member of the Ranunculaceae family. The NS seeds contain volatile and fixed oils, proteins, amino acids, alkaloids, fibers, carbohydrates, minerals, and various vitamins. Thymoquinone (TQ) is among the most active components of NS. Its chemical structure is as follows: $C_{10}H_{12}O_2$, 2-isopropyl-5-methyl 1, 4-benzoquinone [16,17]. It was reported that TQ has antihyperlipidemic and anti-hypercholesterolemic, antioxidative, anti-diabetic, antitumoral and anticarcinogenic, analgesic, anti-inflammatory, antibacterial, anthelmintic, and antifungal properties in the digestive, immune, nervous, respiratory, circulatory, excretory systems, and protective effects on liver and other tissues [9, 11, 18-25].

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The present study aimed to investigate the effects of T and TQ on lung tissues, which were never studied in the literature.

Materials and Methods

Rats and experimental groups

The study was approved by the İnönü University Faculty of Medicine Animal Experiments Ethics Committee (2020/17-4). The study was conducted with the male rats with an average weight of 250 g and bred at the Experimental Animal Production and Research Center of the same institution. To compare the four groups at %95 confidence level (α=0.05) and %80 power (β=0,20) when the effect size is considered to be 0.69, the required minimum sample size for each group is calculated as 8.32 rats were equally divided into Control, T, TQ, T+TQ groups.

T group: 100 mg/kg/day T administration (Sigma-Aldrich-1934-21-0, St. Louis, USA) [26].

TQ group: 50 mg/kg/day TQ administration (Sigma-Aldrich-490-91-5, St. Louis, USA) [27].

T+TQ group: 100 mg/kg/day T + 50 mg/kg/day TQ administration.

T was dissolved in physiological saline and TQ was dissolved in corn oil and administered with oral gavage for 21 days.

Tissue collection and storage

After 21 days, rat lung tissues were removed under anesthesia and excess blood was removed with physiological saline. Lung tissue samples were adequately packaged. The samples were immediately transferred to the deep freezer at -76 °C.

Preparation of the samples for analysis

Lung tissues were quickly weighed on the day of analysis. Phosphate buffer, 9 times the weight of the samples, was added. Samples were homogenized for 1 min. An appropriate homogenate amount was removed for malondialdehyde (MDA) measurement. The samples were placed in a centrifuge to obtain homogenate supernatant. Samples were centrifuged at 600 x g for 25 min to obtain the supernatant. Supernatant samples were employed to determine glutathione (GSH), glutathione peroxidase (GSH-Px), superoxide dismutase (SOD), catalase (CAT), and protein levels.

MDA analysis

The analysis was conducted based on the method developed by Ohkawa et al. and with the Elisa device set to 535 nm [28].

GSH analysis

The analysis was conducted based on the Ellman method and with the Elisa device set to 410 nm [29].

GSH- Px analysis

The analysis was conducted based on the Paglia and Valentine method and with the Elisa device set to 340 nm [30].

SOD analysis

The analysis was conducted based on the method developed by Sun et al. and with the Elisa device set to 560 nm [31].

CAT analysis

The analysis was conducted based on the Aebi and Bergmeyer method and with the Elisa device set to 240 nm [32].

Protein analysis

The analysis was conducted based on the method developed by Lowry et al. and with the Elisa device set to 660 nm [33].

Statistical analysis

Quantitative data are presented as medians, minimums and maximums. Intra-group comparison was conducted with the Kruskal-Wallis test, and Conover method was employed in pairwise comparison. Significance level was accepted as $p \leq 0.05$ in all analyses. The number of samples in each group was 8, so a non-parametric hypothesis testing procedure was used to compare the groups. Descriptive of the quantitative data are presented as medians, minimums and maximums. Independent group comparisons were conducted with the Kruskal-Wallis test, and Conover method was employed in pairwise comparisons. Significance level was accepted as $p \leq 0.05$ in all analyses.

Results

The analysis of the lung tissues revealed differences between the groups. Significant increases were determined in MDA and SOD levels in the T-administration group when compared to all other groups. Also decreases in GSH, GSH-Px and CAT levels were observed in the same group when compared to all other groups. Significant increases were determined in GSH, GSH-Px and CAT levels in the TQ-treatment group when compared to all other groups. Decreases were observed in MDA and SOD levels in the same group when compared to all other groups.

We determined decreases in MDA and SOD levels in the T+TQ group when compared to the T group. Also, we identified significant increases in the decreased GSH, GSH-Px and CAT levels in the T group after TQ administration (Table 1).

Discussion

Lungs are located on the right and left sides of the chest cavity and among the most important organs. They play a key role in the circulation of oxygen and removal of carbon dioxide from the blood. There is a membrane on the outer surface of both lungs. It allows the movement of the organ in the chest cavity. Blood circulation is a key system in human body. Blood circulation should have certain oxygen levels. Due to the connection between the lungs and the heart, the heart is prevented from pumping venous blood. Venous blood is removed in the lungs and healthy oxygen is transferred via blood circulation. Lung health is quite significant to allow the oxygen to reach the blood

Table 1. Oxidant – antioxidant parameters.

Groups	MDA (nmol/gwt)	GSH (nmol/gwt)	SOD (U/g Protein)	CAT (K/g Protein)	GSH-Px (U/g Protein)
Control	125 (113-165)[a]	670 (606-745)[a]	45 (40-49)[a]	29 (19-31)[a]	68 (57-74)[a]
Thymoquinone	126 (99-144)[a]	741 (704-810)[b]	37 (31-46)[b]	41 (35-56)[b]	81 (76-86)[b]
Tartrazine	147 (128-163)[b]	637 (608-642)[c]	51 (45-57)[c]	24 (21-28)[c]	63 (57-68)[c]
Thymoquinone + Tartrazine	135 (99-142)[c]	680 (635-712)[a]	44 (37-46)[a]	32 (25-34)[d]	73 (67-80)[d]
p	<0.001	<0.001	<0.001	<0.001	<0.001

Data are expressed as median (minimum–maximum). There is a statistically significant difference between groups marked with different letters. Abbreviations: MDA, malondialdehyde; GSH, reduced glutathione; SOD, superoxide dismutase; CAT, catalase;, GSH-Px, glutathione peroxidase, gwt, gram wet tissue.

and blood vessels [34]. It was reported that T leads to lung damage and other complications such as asthma [13].

Sasaki et al. investigated the effects of common food additives on mouse tissues. These food additives included Amaranth, Allura Red, New Coccine, Tartrazine, Erythrosine, Phloxine and Rose Bengal. They reported that various doses of these food additives led to DNA damage (Comet Assay) in the stomach, liver, kidney, brain and lung tissues [35]. Ishihara and Kitamura administered T to guinea pigs and investigated its effects on lung tissues. The analysis of the changes in tracheal tissues revealed bronchoconstrictor responses in these tissues, which led to asthma [36]. Hariparsad et al. reported T sensitivity in 10 asthmatic children. 1 mg T was administered orally for 10 days. They observed that there was no change in lung functions after T administration; however, 4 children exhibited histamine sensitivity [37].

Plants are significant nutrients for humans and other living beings. Especially aromatic and medicinal plants are widely consumed in daily life as flavor enhancers and for therapeutic purposes [38-43]. Nigella sativa is among these plants with a long history of consumption. Several studies reported that Nigella sativa had antioxidant, anti-inflammatory, antidiabetic, anticarcinogenic, and immunomodulatory effects. Active ingredients of the plant include thymoquinone, thymo-hydroquinone and di-thymoquinone [44]. Doğru et al. administered 50 mg/kg/day TQ to rats to prevent radiation-induced lung damage. TQ administration improved TOS and OSI oxidative stress parameters when compared to the irradiation group [45]. Sakineh et al. administered 10 mg/kg TQ to rats for 10 days against methotrexate-induced lung damage. They observed improvements in lung tissue histopathology after TQ application when compared to the methotrexate group. They also identified a decrease in MDA and an increase in TAS after TQ administration [46]. Mao et al. developed a TQ protection rat model against Particle Matter 2.5 (PM 2.5)-induced lung damage. They administered 20 and 40 mg/kg TQ for 14 days. They reported that 40 mg/kg TQ administration reduced MDA levels when compared to the PM 2.5 group, increased GSH-Px and SOD levels, and improved histopathologic parameters when compared to 20 mg/kg administration [47]. The findings demonstrated that TQ had protective properties against lung damage. The present study findings were consistent with previous studies.

Conclusion

The impact of T and TQ administration on lung tissues was investigated for the first time in the present study. T led to damages in lung tissue. It could be suggested that these damages were due to oxidative damage induced by the oxidant properties of T. TQ has strong antioxidant properties. Thus, TQ could be consumed to inhibit oxidative stress in T-induced damages. Since this is the first study on the topic, further comprehensive studies are required.

Competing interests

The authors declare that they have no competing interest.

Financial disclosure

There are no financial supports.

Ethical approval

The decision of the ethics committee of Inonu University Faculty of Medicine numbered (2020/17-4).

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