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The effect of tartrazine and thymoquinone on the antioxidant system and lipid peroxidation in brain tissue

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MAIN POINTS

- The relationship between Tartrazine and Thymoquinone in brain tissue was investigated.
- We revealed that Tartrazine induces oxidative stress in brain tissue.
- Thymoquinone has a very strong antioxidant effect and is recommended to be consumed regularly for brain health.

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ABSTRACT

Aim: To investigate the possible effects of harmful Tartrazine and protective Thymoquinone on brain tissue in Wistar albino rats.

Materials and Methods: Thirty-two rats were divided into 4 groups: Control (n=8), Tartrazine (n=8), Thymoquinone (n=8), and a combination of Tartrazine and Thymoquinone (n=8). Tartrazine and Thymoquinone were administered orally via gavage for 3 weeks, and the brain tissues were collected after the 3-week period. Oxidant-antioxidant parameters such as malondialdehyde (MDA), glutathione (GSH), glutathione peroxidase (GSH-Px), superoxide dismutase (SOD), catalase (CAT) were examined.

Results: There were differences between the Tartrazine and all other groups. Tartrazine administration led to a significant increase in MDA and SOD enzymes activity levels in brain tissue. It also caused a significant decrease in reduced GSH, GSH-Px and CAT activities. Thymoquinone administration caused an increase in GSH, GSH-Px enzymes activity, and CAT enzymes activity levels compared to all other groups.

Conclusion: This study examined the effects of tartrazine and thymoquinone on brain tissue found that tartrazine has neurotoxic effects. We believe that the neurotoxicity caused by tartrazine results from oxidative stress, increased oxidant capacity, and decreased antioxidant capacity. Thymoquinone may serve as a neuroprotective agent because it significantly increased antioxidant capacity.

Keywords: Tartrazine, Thymoquinone, Brain, Oxidative stress, Rats

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INTRODUCTION

Tartrazine is an azo dye with the chemical formula of $C_{16}H_9N_4Na_3O_9S_2$. It is commonly used as a yellow dye [1,2]. It enhances the visual appeal of products while maintaining consistent color. Tartrazine (E 102) is a yellow powder employed as a colorant in the food industry. This compound belongs to the triarylmethane group, a class of organic compounds characterized by azo bonds. It exhibits numerous chemical properties and applications in the cosmetics industry, including makeup products, perfumes, and skincare items. It is also utilized in the pharmaceutical and textile industries. As the global population and food demand grow, various physical tools and chemical products have been developed to support food supply. These social changes have increased the demand for processed foods that can be transported from farms to cities while preserving nutritional value and organoleptic properties [3]. The availability of food additives has also expanded due to advancements in the food industry and the rise in processed food production [4,5]. Tartrazine is present in various food items, such as cakes, candies, canned vegetables, cheeses, gums, sausages, ice cream, orange drinks, salad dressings, seasonal salads, desserts, jams, baked goods, snacks, canned fish, ready- to- serve soups, soft drinks, and ketchup [6]. Tartrazine can negatively affect intestinal microbiota, and it is believed to significantly reduce mucus thickness and contribute to colon cancer, obesity, and diabetes [7,8]. It may have carcinogenic effects and trigger allergic reactions. These reactions include angioedema or chronic urticaria, and symptoms such as atopic dermatitis, flushing, abdominal pain, diarrhea, hypotension, and severe anaphylactoid or anaphylactic reactions similar to asthma. It can also induce hyperactivity in children [9]. Studies on experimen-

tal animals have reported that Tartrazine causes hepatotoxicity, nephrotoxicity, reproductive and developmental toxicity, neurotoxicity, and tissue damage [10-18].

Although endemic to the Eastern Mediterranean, Nigella sativa, a member of the Ranunculaceae family, is also commonly found in Turkey and is an annual plant. It contains nearly forty bioactive compounds, the most active of which is thymoquinone ($C_{10}H_{12}O_2$, 2-isopropyl -5-methyl -1,4-benzoquinone). Studies have shown that thymoquinone has antioxidant, anti-inflammatory, immunoregulatory, anticancer, antimicrobial, neuroprotective, hepatoprotective, nephroprotective, gastroprotective, hypoglycemic, antidiabetic, hypolipidemic, and antihistamine properties, and is effective in treating heart and respiratory system diseases, as well as in inhibiting apoptosis [19-25].

In this research, we aimed to investigate biochemical methods the effects of possible neurotoxicity of tartrazine and protective role of thymoquinone administration on rat brain tissues.

MATERIALS AND METHODS

Rats and experimental groups

The study involved 250 ± 25 g male Thirty-two Wistar albino rats bred at the Inonu University Faculty of Medicine Experimental Animal Breeding and Research Center. After approval by the ethics committee (2020/17-4), the rats were randomly divided into four cages, with 8 rats in each group.

Corn oil was given to the control group. Tartrazine 100 mg/kg/day of was given to the Tartrazine group [26]. Thymoquinone 50 mg/kg/day of was given to the Thymoquinone group [27]. A dose of 100 mg/kg/day Tartrazine and 50 mg/kg/day Thymoquinone was given to the Tartrazine + Thymoquinone group. Administrations were given orally by gavage. All doses were one mL and administered at the same time every day for 21 days. Tartrazine was dissolved in physiological serum, Thymoquinone was dissolved in corn oil.

Tissue collection

After the applications treatments, rats were anesthetized with 10 mg/kg Xylazine (Rompun®, Bayer, Topkapı, Turkey) and 40 mg/kg Ketamine (Ketalar, Pfizer, Istanbul, Turkey) were administered intraperitoneally. Brain tissues from the rats were removed under anesthesia. Samples were quickly stored in a freezer at -75°C.

Preparation of the tissues

Brain tissues were quickly weighed on ice packs, and phosphate buffer was added in a volume nine times their weight. This prepared the tissues for homogenization (IKA ultra turrax T 25 basic). The tissues were homogenized for 1 minute, and a sample was taken from this homogenate for malondialdehyde (MDA) measurement. The homogenate was then centrifuged. The supernatant was obtained by centrifuging for 20 minutes at 650 × g. A sample was taken from this supernatant for the analysis of reduced glutathione (GSH), glutathione peroxidase (GSH-Px), superoxide dismutase (SOD), catalase (CAT), and protein levels.

Measurements

MDA

0.25 mL of brain tissue homogenates was pipetted into glass tubes. To each tube, 1.5 mL of 1% H_3PO_4 and 0.5 mL of 0.6% TBA were added. The tubes were then stored in a water bath at 100 °C for 45 minutes. After cooling under tap water, 2 mL of n-butanol was added. The tubes were vortexed and centrifuged at 4400 rpm at 25 °C for 24 minutes. Readings were taken at 535 nm from the upper section of the glass tube [28].

GSH

125 µl of brain tissue supernatant was pipetted into polypropylene tubes, and 125 µl of tricarboxylic acid was added. The tubes were vortexed and centrifuged at 4400 rpm for 15 minutes at +4 °C. Then, 30 µl of the mixture was transferred to another polypropylene tube. 30 µl of DTNB and 235 µl of Na_2HPO_4 were added, and the tubes were gently vortexed. The samples were then removed, and readings were taken at 410 nm [29].

SOD

500 µl of the brain tissue supernatant was pipetted into polypropylene tubes, and 2450 µl of a mixture containing xanthine, Na_2EDTA, NBT, BSA, and Na_2CO_3, along with 50 µl of XO dissolved in $(NH_4)_2SO_4$, was added. The tubes were vortexed thoroughly, then incubated for 25 minutes. One milliliter of $CuCl_2$ was added to this mixture, and the samples were read at 560 nm [30].

CAT

10 µl of the brain tissue supernatant was pipetted into polypropylene tubes. 2990 µl of a mixture of KH_2PO_4, Na_2HPO_4, and H_2O_2 was added, and the tubes were capped and inverted. Readings were taken at 240 nm. The readings were monitored for one minute [31].

GSH-Px

20 µl of brain tissue supernatant was pipetted into polypropylene tubes. 2650 µl phosphate buffer with EDTA, 10 µl reduced glutathione, 10 µl NADPH, 10 µl GSH reductase, and 10 µl NaN_3 were added, and the tubes were mixed thoroughly. Readings were taken at 340 nm. The readings were monitored for three minutes [32].

Protein

12.5 µl of brain tissue supernatant was pipetted into polypropylene tubes. 237.5 µl of distilled water and 250 µl

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Table 1. Oxidant -- antioxidant parameters.

	Control (n=8)	Thymoquinone (n=8)	Tartrazine (n=8)	Tartrazine +Thymoquinone (n=8)	p
MDA nmol/gwt tissue	325 (318-365)[a]	327 (307-347)[a]	577 (498-623)[b]	405 (367-472)[c]	≤ 0.05
GSH nmol/gwt tissue	270 (198-315)[a]	341 (294-450)[b]	207 (177-282)[c]	280 (203-312)[a]	≤ 0.05
SOD U/g protein	15 (10-19)[a]	17 (11-22)[a]	21 (15-37)[b]	14 (11-23)[a]	≤ 0.05
CAT K/g protein	9 (3-11)[a]	17 (9-23)[b]	4 (2-7)[c]	12 (5-19)[d]	≤ 0.05
GSH-Px U/g protein	18 (7-24)[a]	21 (16-26)[b]	13 (5-17)[c]	24 (17-32)[d]	≤ 0.05

MDA, malondialdehyde; GSH, reduced glutathione; SOD, superoxide dismutase; CAT, catalase; GSH-Px, glutathione peroxidase. Data are expressed as median (min-max) (n = 8). gwt; gram wet tissue. Groups with different letters in columns are significantly different from each other ($p \leq 0.05$).

of Alkaline copper were added, and the tubes were vortexed thoroughly. 1 ml of phenol was added, and samples were read at 700 nm [33].

Statistical analysis

Quantitative data are presented as medians; minimums and maximums. Intra-group comparisons were conducted using the Kruskal-Wallis test, with the Conover test applied for pairwise comparisons. The significance level was set at $p \leq 0.05$ for all analyses. To compare the four groups at %95 conf idence 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 eight. Each group had eight samples, so a non-parametric hypothesis test was used to compare the groups. The descriptive statistics of the quantitative data are presented as medians, minimum values, maximum values. Independent group comparisons were performed using the Kruskal-Wallis test, and the Conover test was used for pairwise comparisons. The significance level was accepted as 0.05 in all tests. IBM Statistical Software Package for Social Sciences (SPSS) for Windows version 26.0 (Armonk, NY) and https://biostatapps.inonu.edu.tr were employed in statistical analysis.

RESULTS

The biochemical examination of brain tissues revealed significant alterations between the control, Tartrazine, and Thymoquinone groups. Administration of Tartrazine induced a state of oxidative stress, characterized by a significant elevation in MDA levels and SOD activity compared to the control and Thymoquinone groups. This was accompanied by a marked depletion of endogenous antioxidants, including glutathione (GSH) levels and the enzymatic activities of GSH-Px and CAT. In contrast, Thymoquinone demonstrated a potent antioxidant effect, significantly increasing the levels of GSH, GSH-Px, and CAT, while reducing MDA and SOD activity relative to the other treatment groups. Crucially, the co-administration of Thymoquinone with Tartrazine appeared to ameliorate Tartrazine's detrimental effects. This combination group exhibited significantly reduced MDA and SOD activity and elevated levels of GSH, GSH-Px, and CAT when compared to the group receiving Tartrazine alone (Table 1).

DISCUSSION

Oxidative stress plays a crucial role in brain aging. The brain is the most vulnerable organ to oxidative stress because it has a higher oxygen demand than all other organs. This stress causes significant damage to lipids, proteins, and DNA within brain tissue. Such damage contributes to brain aging, nerve cell death, and declining cognitive functions. Furthermore, oxidative stress is a primary cause of neurodegenerative diseases like Alzheimer's, Parkinson's, and Huntington's disease, all involving damage to brain tissue. Essawy et al. developed a model of 7.5 mg/kg/day Tartrazine-induced neurotoxicity over 4 weeks and administered 10 mg/kg/day Melatonin as a protective agent. When examining the rats' brain tissues at the end of the study, they reported that Tartrazine exposure reduced GSH, GSH-Px enzyme activity, and CAT enzyme activity levels compared to other groups [17]. Woopara et al. administered 10 mg/kg of Tartrazine for six weeks and studied the rats' brain tissues. After this period, they observed increased MDA levels and a significant decrease in GSH levels in the Tartrazine group compared to the control [34]. Albasher et al. gave pregnant rats 2.5 mg/kg, 5 mg/kg of Tartrazine through drinking water during pregnancy and for 15 days after birth. Male rats were then selected for brain tissue analysis. They found that Tartrazine increased MDA levels and decreased GSH levels in a dose-dependent manner compared to the control group [35]. Bhatt et al. observed increased MDA levels and decreased CAT enzyme activity levels in brain tissue after administering 7.5 mg/kg of Tartrazine for 21 days [36]. They concluded that Tartrazine raises the oxidant capacity in brain tissue, inducing oxidative stress a key factor in causing nerve cell death and damage in various neurodegenerative and nervous system diseases. They emphasized that this damages brain tissues. Our findings align with these studies, showing that Tartrazine elevates the oxidant capacity and reduces antioxidant capacity in the brain, leading to oxidative stress.

Brain tissue is highly vulnerable to oxidative stress because of its need high oxygen demand, lipid content, limited antioxidant capacity. In the brain, the primary defense against oxidative stress is provided by the antioxidant enzyme system [superoxide dismutase (SOD), glyoxalase, glutathione reductase, glutathione peroxidase, and catalase], along with low molecular weight antioxidants such as glutathione, uric acid, ascorbic acid, and melatonin [37]. Recently, research has focused on

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antioxidants capable of resisting the development of oxidative stress during neurodegenerative processes in the brain. One promising natural antioxidant from plants is thymoquinone [38]. Shrief et al. gave 20 mg/kg of thymoquinone to rats for 4 weeks as a prophylactic in an $AlCl_3$-induced neurotoxicity model. After 4 weeks, they observed a decrease in brain MDA levels and a significant increase in GSH levels compared to the $AlCl_3$ group [39]. Imam et al. administered 10 mg/kg of thymoquinone as a prophylactic in a cypermethrin-induced neurotoxicity model for 14 days. They noted an increase in GSH levels and a decrease in MDA levels in the thymoquinone group compared to controls [40]. Our findings align with these results. Thymoquinone significantly enhances the brain's antioxidant capacity, mainly by increasing GSH, GSH-Px enzyme activity, CAT enzyme activity levels. Additionally, thymoquinone reduces MDA levels, an indicator of oxidative capacity. Therefore, it helps prevent oxidative stress by decreasing oxidant capacity and boosting antioxidant defenses.

CONCLUSION

Tartrazine caused oxidative stress in brain tissue which led to damage in the brain. Thymoquinone administration resulted in a significant increase in antioxidant capacity. It could serve as a protective agent against Tartrazine-induced brain damage.

Ethics Committee Approval: The study was approved by the İnönü University Faculty of Medicine Animal Experimentation Local Ethics Committee (HADYEK) (no: 2020/17-4).

Informed Consent: Not required for this study.

Peer-review: Externally peer-reviewed.

Conflict of Interest: The authors declare that they have no conflict of interest.

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