The Impact of Tartrazine and Thymoquinone Administration on Rat Liver

Nursena Demircigil
İnönü Üniversitesi Tıp Fakültesi: Inonu Universitesi Tip fakultesi
Mehmet Gul
İnönü Üniversitesi Tıp Fakültesi: Inonu Universitesi Tip fakultesi
Nurcan Gokturk
İnönü Üniversitesi Tıp Fakültesi: Inonu Universitesi Tip fakultesi
Elif Kayhan Kustepe
İnönü Üniversitesi Tıp Fakültesi: Inonu Universitesi Tip fakultesi
Harika Gozukara Bag
İnönü Üniversitesi Tıp Fakültesi: Inonu Universitesi Tip fakultesi
Mehmet Erman Erdemli (ermanerdemli@hotmail.com)
Inonu Universitesi Tip fakultesi https://orcid.org/0000-0003-4596-7525

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Abstract

The current study aimed to observe the toxic effects of Tartrazine, a commonly used dyestuff in industries and foods, on the liver, and investigate whether this toxicity could be eliminated with Thymoquinone coadministration. The study was conducted on 32 200-250 g male Wistar albino rats, procured from İnönü University Experimental Animals Breeding and Research Center. The rats were randomly assigned to 4 equal groups: Control group, Thymoquinone group, Tartrazine group, Thymoquinone + Tartrazine group. The trials were conducted for 21 days. Then, rat liver tissue and blood samples were obtained and biochemical and histopthological examinations were conducted on the samples. Tartrazine administration increased the oxidant and oxidative stress index parameters in the liver tissue, decreased the antioxidant parameters, led to histopathological problems and inflammation in the serum samples. Thymoquinone administration increased the antioxidant parameter levels in liver tissue and decreased serum inflammation levels ($p<0.001$). Thymoquinone and Tartrazine coadministration reduced the adverse biochemical and histopathological effects of Tartrazine. Tartrazine caused hepatotoxicity via cellular damage in the liver tissue and led to inflammation. Thymoquinone, on the other hand, improved antioxidant and anti-inflammatory effects. We recommend daily consumption of black cumin and its active ingredient, Thymoquinone to reduce Tartrazine toxicity.

Introduction

Tartrazine, which was indicated as the food additive E102 by the European Union, is a synthetic food dye employed in the food industry. It was first isolated from bitumen by the German chemist J. H. Ziegler in 1884 (Mehedi et al. 2009; Mittal et al. 2007). Tartrazine is an orange-yellow substance and could be in powder or granular form. It is produced with the synthesis of petroleum products. Tartrazine is a water-soluble and frequently preferred coloring used in several products due to the low cost. It is commonly used in puddings, bakery products, chips, ice cream, beverages, dairy products, meat and fish products, and confectionery among others. Tartrazine is not used only as a food dye, but also to color several personal care products (soap, moisturizer, toothpaste, shampoo, and hair care products) and certain drugs (Walton 1999; Mpountaukos et al. 2010).

It was reported that the primary metabolism mechanism for Tartrazine in nutrients is bacteria in humans, rats and rabbits after oral intake. Sulfanilic acid and aminopyrazolone are the main metabolites of Tartrazine (Chung et al. 1992, Jones et al. 1964). It was suggested that the azo dyes in Tartrazine could have mutagenic, carcinogenic and toxic effects due to the reduction of the biotransformation effect of the azo bond (Soares et al. 2015; Amin et al. 2010; Velioglu et al; 2019, Erdemli et al. 2021).

Thymoquinone, which is the main bioactive component in *Nigella Sativa* essential oil, is a volatile monoterpene quinone with a molecular weight of 164.2 g/mol in the form of dark yellow crystals (Ghosheh et al. 1999). It was reported that thymoquinone has antioxidant, immunomodulatory, hepatoprotective, anticarcinogen, gastroprotective, anti-inflammatory, hypoglycemic antimicrobial, nephroprotective, antidiabetic, neuroprotective, hypolipidemic, antihistaminic effects, and affects heart

and respiratory system diseases and apoptosis. It was also reported that it could be a natural remedy in autoimmune diseases (Danaei et al. 2019; Abdel-Daim et al. 2020; Abd Al Haleem et al. 2021; Hashem et al. 2021; Erdemli et al. 2020).

In the current study, the possible adverse effects of tartrazine on liver, and whether thymoquinone could alleviate the toxic effect of tartrazine if it exists were analyzed for the first time in the literature.

Materials And Methods

Procurement and care of the rats

Our study was conducted with 32 Wistar albino male rats procured from İnönü University Experimental Animal Breeding and Research Center. Rats were fed ad libutum with standard pellet feed and tap water throughout the experiments.

Experimental Groups

Group 1: (Control group): Corn oil administration.

Group 2: (Tartrazine group): 100 mg/kg/day tartrazine was administered (Sigma-Aldrich-1934-21-0, St. Louis, USA) (Balta et al. 2019).

Group 3: (Thymoquinone group): 50 mg/kg/day Thymoquinone was administered (Sigma-Aldrich-490-91-5, St. Louis, USA) (Kong et al. 2015).

Group 4: (Tartrazine + Thymoquinone group): 100 mg/kg/day tartrazine + 50 mg/kg/day thymoquinone were administered.

Tartrazine was applied by gavage after it was dissolved in physiological saline and thymoquinone was dissolved in corn oil and 1 ml/kg/day solution was administered to each rat. The solutions were administered at the same time every day for 21 days.

Collection Of The Samples And Preparations

After the experiments, the abdominal region of the rats were opened under anesthesia (xylazine and ketamine) with the protocol prescribed by the ethics committee, and blood samples were obtained from the heart tissue and transferred into adequate tubes. Liver tissues were incised and washed with physiological saline to remove the excess blood. Certain liver tissues were placed in sterile containers and quickly frozen to -80°C for biochemical analyzes. The rest of the liver tissues were placed in containers that included 10% formol solution for histopathological analyses.

Biochemical Analysis

Liver tissues were removed from − 80°C freezer and quickly weighed before thawing. Phosphate buffer that equaled to 9 times the tissue weight was added. They were homogenized at 15000 rpm for 1 minute at + 4°C (IKA, Germany). Malondialdehyde (MDA) levels were determined with these homogenates. The tissue homogenates were centrifuged at 4000 rpm for 25 minutes at + 4°C to obtain the supernatants. Glutathione (GSH), superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), catalase (CAT), total oxidant status (TOS), total antioxidant status (TAS), oxidative stress index (OSI), and protein levels were determined on these supernatants. Blood samples were centrifuged at 600g at 4°C for 20 minutes to obtain the serum, and the serum was used to determine tumor necrosis factor alpha (Tnf-α) and interleukin-6 (IL-6).

Mda Analysis

0.25 ml tissue homogenate was mixed with 1.5 ml 1% H3PO4 and 0.5 ml 0.6% thiobarbituric acid. The product was heated to 30º to completely dissolve the TBA. The mixture was heated at 100ºC for 45 minutes. Then, the formation of the red color was observed, and then the tubes were cooled in tap water and 2ml n-butanol was added to each sample. Each tube was vortexed for 5 minutes. Then, the samples were centrifuged for 25 minutes at 5000 rpm at 25ºC. 25µl supernatant (n-butanol phase) was carefully collected and transferred to individual quartz microplate wells. Readings were conducted at 535 nm. The findings are presented as nanomole/gram wet tissue (Ohkawa et al. 1979).

Gsh Analysis

The supernatant was deproteinized with tricarboxylic acid (TCA). 125 µl TCA was added to 125 µl sample. After the mixture was vortexed, it was centrifuged for 20 minutes at 5000 rpm and + 4ºC to obtain the protein-free supernatant. 29 µl deproteinized sample, 29 µl DTNB and 235 µl Na_2HPO_4 were added to each microplate well separately, gently vortexed and kept for 5 minutes. Readings were conducted at 410 nm within 5 minutes. The findings are presented as nanomole/gram wet tissue (Ellman 1958).

Sod Enzyme Activity

SOD enzyme activity is determined with the measurement the intensity of this color formed by the reduction of NBT by superoxide radicals (xanthine + xanthine oxidase system) with the spectrophotometer. The reduction forms a blue colored formazan with a maximum absorbance at 560 nm. In the absence of enzyme, this reduction is maximal, and a dark blue color is observed. In the presence of SOD, the enzyme converts the superoxide radical to hydrogen peroxide; and thus, the reduction of NBT is lower and no blue formazan is observed or the intensity of the color is quite light.

SOD activity was calculated based on the absorbance of the blue colored formazan at 560nm. The results are presented as U/mg protein (Sun et al. 1988).

Cat Enzyme Activity

Hydrogen peroxide (H_2O_2) is a substance that provides absorbance in the UV spectrum and its maximum absorbance wavelength is 240 nm. A decrease in absorbance is observed at 240 nm due to the breakdown of the added hydrogen peroxide into water and oxygen by catalase. This decrease in absorbance was recorded for 1 min via kinetic readings and the enzyme activity was measured. The enzyme activity is presented as K/g protein (Aebi 1974).

Gsh-px Activity

GSH-Px is an enzyme that catalyzes the conversion of hydrogen peroxide to water by reduced glutathione. After the reaction, reduced glutathione is converted to the oxidized form. For the conversion of another hydrogen peroxide into water, the oxidized glutathione should be converted back to the reduced form. This occurs in the presence of reduced NADP and reduced glutathione in the medium. Then, reduced NADP is converted into oxidized NADP, while oxidized glutathione is converted into reduced glutathione. The maximum absorbance of the reduced NADP is at 340 nm. The absorbance drops at 340nm as glutathione reductase catalysis continues, since the reduced NADP is not converted into the oxidized form. GSH-Px activity is calculated by recording the decrease in absorbance for 3 min. The findings are presented as U/mg protein (Paglia and Valentine 1967).

Protein Analysis

Tissue protein content is required to calculate enzyme activities. The Lowry method was used to determine the tissue protein content. The absorbance of the resulting color was measured at 660 nm in the spectrophotometer to determine the protein content (Lowry et al. 1951).

Tas Analysis

Rel Assay brand kit (Rel Assay Diagnostics, Gaziantep Turkey) was used to determine the TAS. The measurement is based on the discoloration of the antioxidant molecules. Sequential steps were conducted based on the kit instructions. TAS was determined with the measurement of the absorbance at 660 nm in the device set for 37ºC as specified in the kit. The findings are presented as mmol Trolox Equiv./l (Erel 2004).

Tos Analysis

TOS Rel Assay brand kit (Rel Assay Diagnostics, Gaziantep Turkey) was employed to determine the TOS. Ferric ions form a colored chromogenic solution when the oxidants in the sample convert the ferrous ion chelator complexes into ferric ions. TOS is determined with the measurement of the absorbance of the colored complex at 530 nm at 25°C as specified in the kit. The findings are presented as μmol H_2O_2 equiv/l (Erel 2005).

Osi Analysis

The OSI was calculated with the formula OSI (arbitrary unit) = TOS (μmol H_2O_2 eqv/L) / TAS (mmol Trolox eqv/L) X 10. Results are presented as arbitrary units (AU).

Tnf- α And Il-6 Analysis

Bioassay Technology Laboratory ELISA Kit was employed to determine the serum interleukin-6 and TNF-α levels. All reagents were at room temperature before the tests, and the tests were performed at room temperature. Sample and ELISA reagent were transferred to the wells, and these were incubated for 1 hour at 37°C. Then, they were washed five times in the microplate washer with the lavage solution. Substrate solutions A and B were added and incubated at 37°C for 10 minutes. Then, the stop solution was added, and the color change was observed. The readings were conducted within ten minutes. The findings are reported as ng/L.

Histopathological Analysis

For histopathological analysis, liver tissue samples were fixed in 10% formaldehyde for 48 hours. Then, liver tissue samples were dehydrated through an incremental ethanol series (50%, 70%, 80%, 96%, Absolute). The liver tissue samples were then transparentized through xylene series and embedded in paraffin blocks after they were passed through melted paraffin series at 62°C for infiltration. 6 μm thick sections were obtained from the paraffin blocks with a microtome and placed on slides (Bancroft and Gamble 2002). They were stained with hematoxylin–eosin (H-E) and anti-caspase-3 [Cleaved-CASP3p17 (D175) Polyclonal Antibody] (Elabscience, Texas, USA) for immunohistochemical (IHC) analysis. Stained sections were examined with Nikon Eclipse Ni-U light microscope, Nikon DS-Fi3 microscope camera and Nikon NIS-Elements Documentation 5.02 image analysis program (Nikon Corporation, Tokyo, Japan).

Histopathological changes in the liver sections stained with hematoxylin–eosin (inflammatory cell infiltration, necrosis, periportal edema, vascular congestion) were scored between 0 and 3 (0; absent, 1; mild-rare, 2; moderate, 3; severe- common), where the maximum histopathological score was 12.

Immunohistochemistry Analysis

Cleaved caspase-3 expression was considered as an indicator of apoptosis in liver tissue. Sections stained for immunohistochemical analysis were placed on polylysine-coated slides. Sections were initially deparaffinized. Then, they were heat-treated in the retriever (Retriever 2100) (Aptum, Southampton, UK) for 15 min with citrate buffer with a pH of 7.6 (Thermo Scientific, Fremont, CA) for retrieval of the antigens. After the sections were cooled to room temperature for 20 minutes, they were first washed with distilled water and then with phosphate buffered saline (PBS) for 1–2 minutes. Sections were drawn using a hydrophobic pen and lined up on the platform and treated with 3% hydrogen peroxide for 10 minutes to inhibit endogenous peroxidase activity, then washed with PBS. Sections were incubated with protein-V blocking reagent (Thermo Scientific) for 5 minutes.

Sections were incubated with 1:200 diluted primary rabbit polyclonal cleaved caspase-3 antibody (Cleaved-CASP3p17 (D175) Polyclonal Antibody) (Elabscience, Texas, USA) for 1 hour, then rinsed in PBS, and incubated with biotinylated goat anti-polyvalent secondary antibody for 10 minutes and transferred into PBS. It was then incubated with streptavidin peroxidase (HRP) for 10 minutes and transferred into PBS. The polyvalent HRP kit (Thermo Scientific) was employed in compliance with the manufacturer's instructions. Finally, sections were treated with chromogen (AEC; Thermo Scientific) + substrate buffer (AEC) (Thermo Scientific) for a maximum of 15 minutes. After they were washed with PBS and distilled water, counterstaining was conducted with Mayer's hematoxylin for 1 minute. Sections were rinsed in tap water and then distilled water and covered with a water-based sealer and coverslip (Thermo Scientific, Cheshire, UK).

The caspase-3 immunoreactivity H score (H Score = Pi (i + 1), Pi, is the percentage of stained cells in each density category (0-100%) in sections stained with anti-caspase-3 in the immunohistochemical method, and i denotes weak (i = 1), moderate (i = 2), or strong staining (i = 3) (Budwit-Novoty et al. 1986).

Statistical Method

Numerical data were summarized with median, minimum and maximum values. Kruskal-Wallis test was used for independent group comparisons and Friedman test was used for dependent group comparisons. After both universal tests, pairwise comparisons were made with the Conover method. The significance level was accepted as 0.05 in all tests.

Results

Biochemical

No significant difference was determined between the changes in rat weight based on the groups. Weight gain was observed in rats in all groups in each week (Table 1).

It was determined that the oxidative stress parameters MDA and SOD levels increased and GSH, GSH-Px and CAT levels decreased in the liver tissue of the Tartrazine group rats when compared to all other

groups. TOS increased and TAS decreased in liver tissue of the Tartrazine group rats when compared to all other groups. Tartrazine led to an increase in Tnf-α and IL-6, the inflammatory agents, in rat serum when compared to all other groups. Thymoquinone administration led to an increase in CAT, GSH, GSH-Px, and a decrease in MDA and SOD levels. Thymoquinone administration increased the TAS levels and decreased the TOS in rat liver tissue when compared to all other groups. Serum Tnf-α and IL-6 levels decreased with thymoquinone administration when compared to all other groups. Tartrazine + Thymoquinone administration improved oxidative stress parameters, OSI markers and inflammation factors when compared to the tartrazine group (Tables 2, 3 and 4).

Histopathology

It was determined that the hepatocyte cords, sinusoids, central vein and portal areas in liver parenchyma exhibited normal histology after hematoxylin-eosin staining in the control and Thymoquinone groups. Liver lobules presented the usual structure, including radially organized hepatocyte cords and sinusoids around the central vein. The portal triad connective tissue and related vascular structures and bile ducts were open and presented normal histological structures (Fig. 1, 2). In the hematoxylin-eosin-stained liver sections in the Tartrazine group exhibited necrosis of various length in liver parenchyma. Inflammatory cell infiltration was observed in the regions of parenchymal necrosis, and portal and periportal regions. Apoptotic hepatocytes with dark eosinophilic cytoplasm and heterochromatic pycnotic nuclei were identified in the parenchyma. Congestion was noted in vena porta branches in the portal regions. The mall distance between the portal region connective tissue and peripheral hepatocytes was significantly larger due to periportal edema. Furthermore, periductal edema was observed around the bile ducts in the portal region. Damaged and degenerated cholangiocytes were noted in the bile duct (Fig. 3). In the hematoxylin-eosin-stained liver sections of the rats in the Tartrazine + Thymoquinone group, rare focal necrosis, minimal inflammatory cell infiltration and periportal edema were observed in the parenchyma. However, the extent of these damages was significantly reduced when compared to the Tartrazine group (Fig. 4).

Caspase-3 immunoreactivity was similarly weak and minimal in the immunohistochemical anti-caspase-3 staining administration regions in the control and Thymoquinone groups. In these groups, caspase-3 immunoreactivity H score was 1. Intense and prevalent caspase-3 immunoreactivity was observed in the same regions in the Tartrazine group. Tartrazine group caspase-3 immunoreactivity H score 180. The caspase-3 immunoreactivity staining in the regions in the Tartrazine + Thymoquinone group was generally low to moderate intensity. The Tartrazine group exhibited a caspase-3 immunoreactivity H score of 60 (Table 5).

Discussion

Currently, food dyes are frequently used to improve the attractiveness and aesthetics of the food products such as confectionery products. Due to the advances in the food industry and consumer trends that

preferred packaged food items, it became almost impossible to avoid food dyes. It became very difficult to prevent the use of these substances, which could induce hyperactivity, allergic reactions, and asthma, especially among the children. Tartrazine, which is common food coloring, could have toxic effects, inducing oxidative stress, which could lead to neurotoxicity, nephrotoxicity and hepatotoxicity (Hımrı et al. 2011, Balta et al. 2019).

Amin et al. investigated the effects of the Tartrazine and carmoisine on biochemical parameters associated with kidney and liver functions and oxidative stress markers in young male rats, and administered two different doses (500mg/kg/bw and 100mg/kg/bw) of tartrazine to rats orally for thirty days. They reported that SOD, GSH and CAT levels decreased, MDA levels increased significantly in the high-dose group when compared to the control (Amin et al. 2010). In a study where oxidative stress and hepatotoxicity induced by tartrazine was analyzed in male rats, 7.5mg/kg tartrazine was administered to rats for 90 days. The analysis of the rat liver tissues after 90 days revealed MDA and total protein levels increased and SOD, GPX, GSH and CAT levels decreased significantly in the tartrazine group when compared to the control group that was fed a normal diet. Furthermore, they reported histopathological changes in liver tissue (El-Desoky et al. 2017). In a study conducted by Velioğlu et al. 500 mg/kg/day tartrazine was administered to rats orally for 21 days. The comparison of the tartrazine and control groups revealed that SOD, MDA, and TOS levels significantly increased and CAT, TAS and GSH levels decreased in the liver tissues of the tartrazine group, leading to the histopathological damages in the liver tissue (Erdemli et al. 2021). Abd-Elhakim et al. conducted a study on rats and administered 75 mg/kg/bw Tartrazine orally for 90 days. Significant increases were observed in mRNA levels and immunohistochemical localization of collagen 1-a, TGFβ-1 and caspase-3. Furthermore, significant increases were observed in AST, ALP, MDA, creatinine and urea levels and significant decreases were observed in SOD, CAT and GSH enzyme levels in the kidney and liver tissue when compared to the control group. In the histological examination, hepatocytes, apoptotic hepatocytes, and periportal fibrosis with tubular necrosis were observed (Abd- Elhakim et al. 2019).

Thymoquinone is a phenolic compound found in the *Nigella Sativa* plant seed oil, and it is traditionally used to treat several diseases due to high antioxidant properties. In vivo and in vitro studies suggested that thymoquinone could have anti-inflammatory, antioxidant antimicrobial and anticarcinogenic properties (Shahid et al. 2021, Abd Al Haleem et al. 2021). Erdemli ME et al. to investigate the changes in liver tissues induced by thymoquinone administration against 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD)-induced hepatotoxicity, 50 rats were divided into five groups, and the administration was maintained for thirty days. The study was conducted on corn oil, TCDD, thymoquinone, and TCDD + Thymoquinone groups. They reported that TCDD administration led to histopathological changes such as thickening of the Glisson's capsule in liver, intracytoplasmic vacuolization, sinusoidal expansion, vascular and sinusoidal occlusion and inflammatory cell infiltration in hepatocytes based on biochemical and histopathological analyzes. CAT, GSH, SOD and TAS levels increased, and MDA, TOS, ALT, AST, and ALP levels decreased in the thymoquinone treatment group when compared to all other groups (Erdemli et al. 2018). In a 28-day study where the protective properties of thymoquinone against arsenic-induced hepatotoxicity were investigated in rats, it was reported that SOD, CAT, GSH-Px and GSH levels

significantly decreased after arsenic exposure, and these parameters significantly improved after thymoquinone treatment (Al Aboud et al. 2021). In the study by Mabrouk A et al., the potential protective properties of thymoquinone were investigated in Pb-induced liver damage. Adult male rats in the Pb group (2000 ppm Pb acetate via drinking water) were administered 5 mg/kg/day thymoquinone for 5 weeks. The findings demonstrated that Pb exposure increased hepatic Pb content, damaged hepatic histological structure (necrotic foci, hepatic filament disorder, hypertrophic hepatocytes, cytoplasmic vacuolization, cytoplasmic loss, chromatin condensation, mononuclear cell infiltration, congestion, centrilobular swelling) and altered liver functions. They also reported that Pb administration reduced total antioxidant status and increased lipid peroxidation in liver. It was reported that thymoquinone supplement significantly ameliorated the adverse effects induced by Pb (Mabrouk et al. 2016). In a study where hepatorenal protection mechanisms of thymoquinone were investigated in methotrexate-induced rat toxicity, the authors administered oral thymoquinone (10 mg/kg) for 10 days. The comparison of the methotrexate and thymoquinone + methotrexate groups revealed that the methotrexate-induced decreases in the liver and kidney GSH and CAT levels approached control group levels and the increased MDA and TNF-α levels decreased (El- Sheikh 2015).

In the current study, the analysis of the efficacy of thymoquinone against food dye tartrazine toxicity in the liver tissue revealed similar findings when compared to other reports in histological, immunohistochemical and biochemical analyses. The findings demonstrated that Tartrazine led to hepatotoxicity via oxidative stress and inflammation, while Thymoquinone minimized Tartrazine-induced hepatotoxicity due to strong antioxidant and anti-inflammatory properties. Exposure to food coloring substances has increased with the prevalence of take-home food products. Thus, consumption of the common substances in these food items such as Tartrazine could not be avoided. To eliminate the toxic effects of Tartrazine or at least to minimize these hepatoxic effects, we recommend daily consumption of *Nigella sativa* and its active component, Thymoquinone, when possible.

Declarations

Author contribution Nursena Demircigil and Nurcan Gokturk studied biochemical analysis, Mehmet Erman Erdemli, Nursena Demircigil and Nurcan Gokturk designed the study and collected the tissues, Mehmet Gul and Elif Kayhan Kustepe performed the histological examination of the liver tissues, Harika Gozukara Bag calculated the biochemical and histological results, Mehmet Erman Erdemli was a major contributor in writing the manuscript.

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Availability of data and materials The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Ethical approval and and consent to participate The study was approved by the Experimental Animals Ethics Committee of Inonu University, Faculty of Medicine (Protocol No: 2020/17-4).

Consent to publish Not applicable

Conflict of interest The authors declare no competing interests.

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Tables

Table 1: Weekly weight changes of rats

Groups	1st Week	2 nd Week	3 rd Week
Control	295.5 (280-307) [a]	315.5 (293-342) [b]	328.5 (302-352) [c]
Thymoquinone	274 (249-290) [a]	295 (267-315) [b]	303 (275-320) [c]
Tartrazine	250.5 (232-271) [a]	271 (243-282) [b]	281 (252-290) [c]
Thymoquinone + Tartrazine	268.5 (241-298) [a]	274 (243-314) [b]	288 (246-319) [c]
p	<0.001	<0.001	<0.001

Table 2: Oxidant – antioxidant parameters of rats

Groups	MDA (nmol/gwt)	GSH (nmol/gwt)	SOD (U/mg Protein)	CAT (K/mg Protein)	GSH-Px (U/mg Protein)
Control	116.9 (108.8-126.4) [a]	1089 (1008-1351) [a]	1.9 (1.2-3.1) [a]	48.7 (38.2-60.2) [a]	262 (246.307) [a]
Thymoquinone	102 (87.72-117.6) [b]	1349 (1062-1972) [b]	0.9 (0.4-2.2) [b]	65.4 (62.3-67.9) [b]	290 (262-325) [b]
Tartrazine	132 (121-138.8) [c]	885 (809-947) [c]	2.9 (2.3-4) [c]	30.3 (25.-38.9) [c]	230 (204-262) [c]
Thymoquinone + Tartrazine	112.2 (95.2-129.8) [a,b]	1086 (932-1198) [a]	1.5 (0.7-2.2) [b]	37.8 (34.5-47.2) [d]	242 (213-270) [d]
p	<0.001	<0.001	<0.001	<0.001	<0.001

Table 3: Oxidative stress index parameters of rats

Groups	TOS ($\mu molH_2O_2$ Eqv/l)	TAS (mmol Trolox Eqv/l)	OSI (AU)
Control	17.8 (16.4-21.8) [a]	2.25 (2.1-2.27) [a]	81.3 (75-96.2) [a]
Thymoquinone	14.9 (13-19) [b]	4.07 (3.86-4.98) [b]	37.8 (26.7-39.2) [b]
Tartrazine	28.2 (25.9-29.4) [c]	1.99 (1.82-2.08) [c]	140 (124.9-162) [c]
Thymoquinone + Tartrazine	16.8 (15.1-21.2) [a]	2.13 (1.84-2.31) [d]	82.2 (74.6-95.4) [a]
p	<0.001	<0.001	<0.001

Table 4: Inflammation parameters of rats

Groups	TNF-α (ng/L)	IL-6 (ng/L)
Control	82.6 (69.4-112.5) [a]	63.9 (46.4-79.7) [a]
Thymoquinone	76.4 (74.1-96) [b]	57.3 (41.1-69.1) [b]
Tartrazine	104.5 (78.8-116) [c]	83.6 (67.8-89.9) [c]
Thymoquinone + Tartrazine	89 (73.1-110.5) [d]	73.5 (57.2-86) [d]
p	<0.001	<0.001

Table 5: Histopathology and Caspase - 3 damage scores of rats

Groups	Inflammatory cell infiltration	Necrosis	Periportal edema	Vascular congestion	Total Damage score	Caspase-3 Damage score
Control	0 (0-0) [a]	0 (0-0) [a]	0[a] (0-0) [a]	0[a] (0-0) [a]	0 (0-0) [a]	1 (1-10) [a]
Thymoquinone	0 (0-0) [a]	0 (0-0) [a]	0 (0-0) [a]	0 (0-0) [a]	0 (0-0) [a]	1 (1-10) [a]
Tartrazine	1 (1-2) [b]	1 (1-2) [b]	1 (1-2) [b]	2 (1-2) [b]	5 (4-7) [b]	180 (160-240) [b]
Thymoquinone + Tartrazine	0 (0-1) [c]	0 (0-1) [c]	1.5 (0.7-2.2) [c]	0.5 (0-1) [c]	1 (1-3) [c]	60 (50-80) [c]
p	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Figures

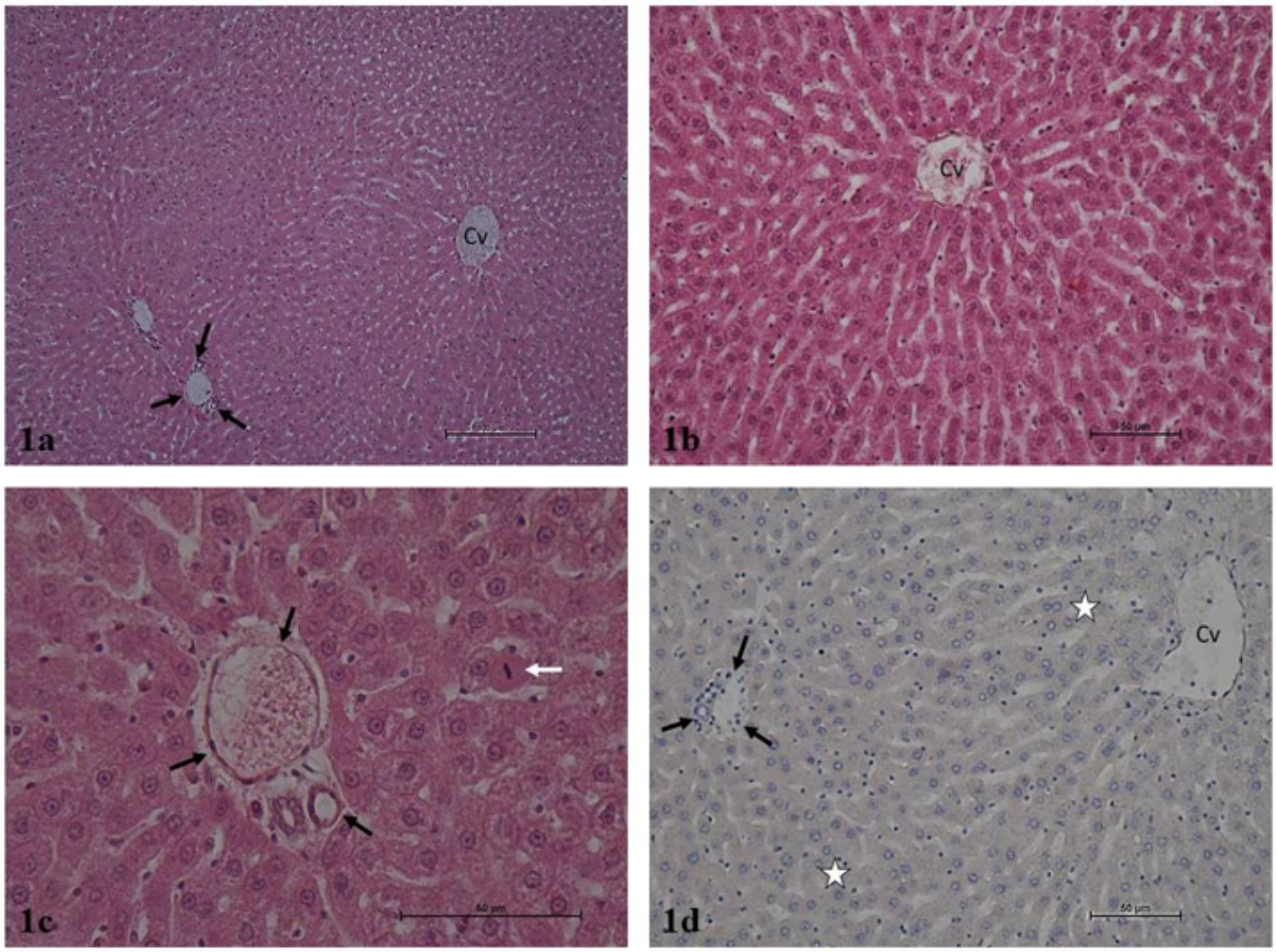

Figure 1

a. Control group. Liver parenchyma in normal histological structure. Central vein (Cv), portal region (arrows). H-E, x10

b. Control group. Liver parenchyma in normal histological structure. Central vein (Cv), portal region (arrows). H-E, x40

c. Control group. Liver parenchyma in normal histological structure. Mitosis figure (white arrow), portal region (black arrows). H-E, x40

d. Control group. Liver parenchyma, caspase-3 immunoreactivity negative (asterisk), central vein (Cv), portal region (arrows). H-E, x20

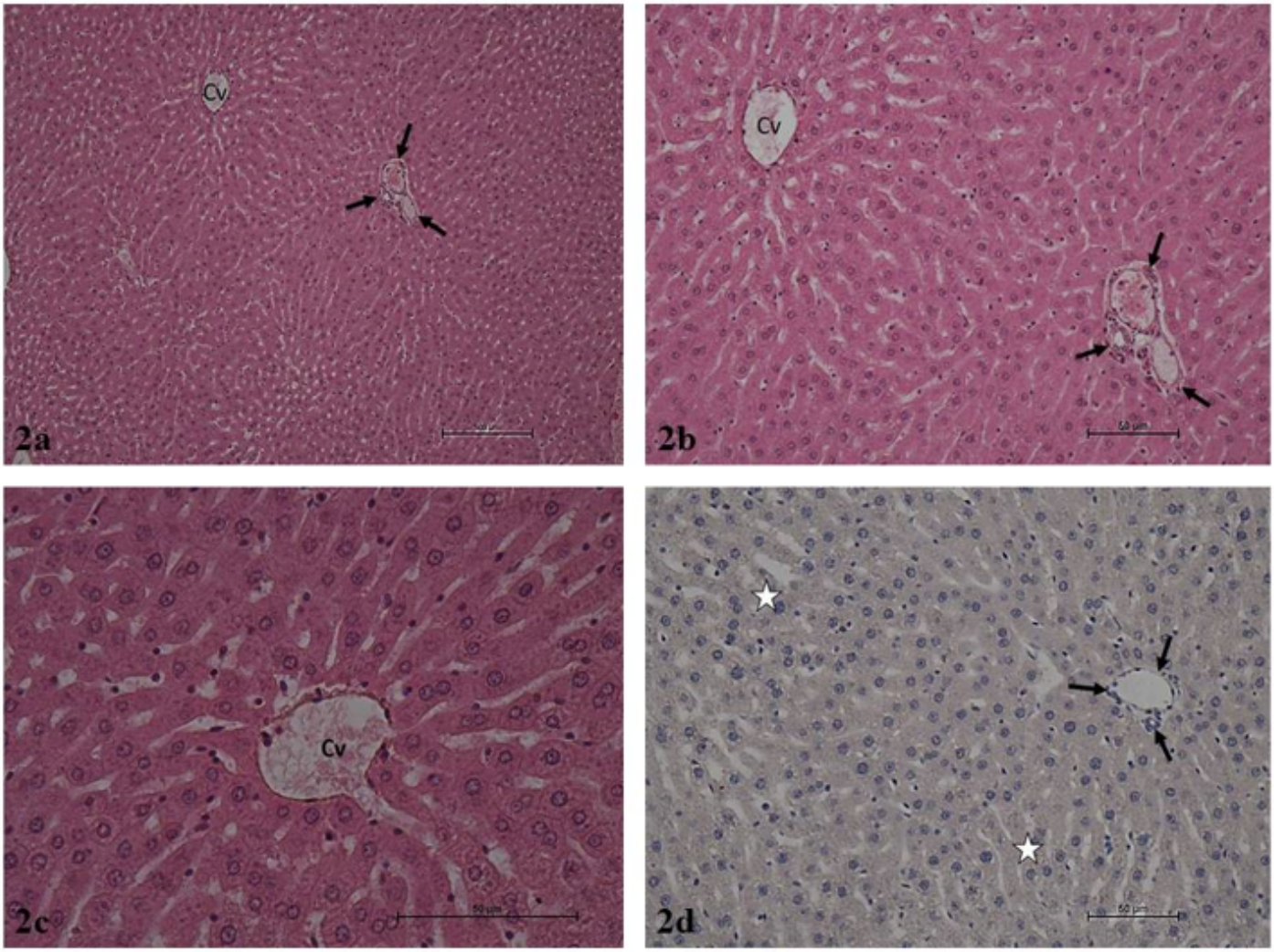

Figure 2

2a. Thymoquinone group. Liver parenchyma in normal histological structure. Central vein (Cv), portal region (arrows). H-E, x10

2b. Thymoquinone group. Liver parenchyma in normal histological structure. Central vein (Cv), portal region (arrows). H-E, x20

2c. Thymoquinone group. Liver parenchyma in normal histological structure. Central vein (Cv). H-E, x40

2d. Thymoquinone group. Liver parenchyma, negative caspase-3 immunoreactivity (asterisk), portal area (arrows). IHC, x20

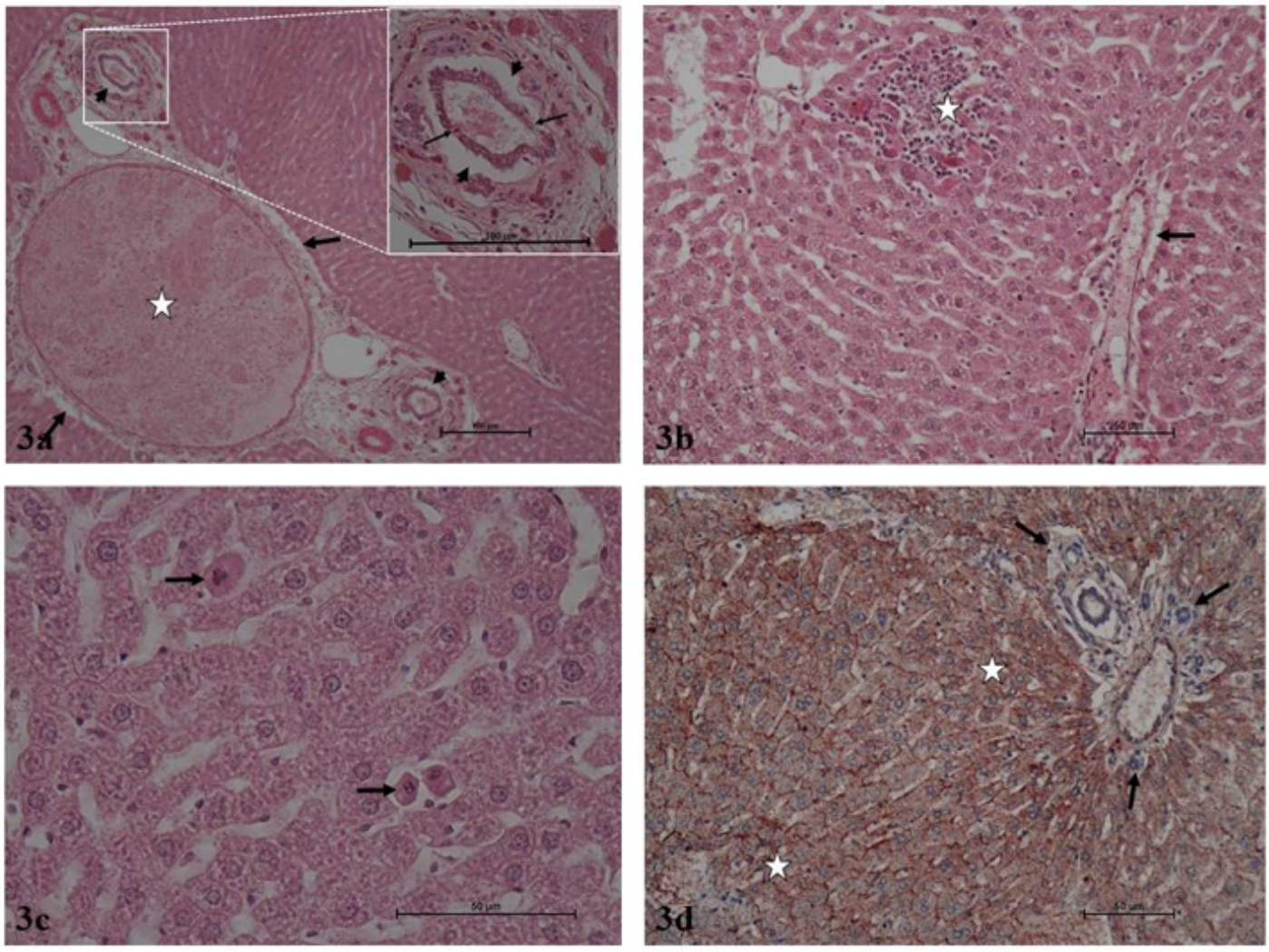

Figure 3

a. Tartrazine group. Large image. Portal vein lumen congestion (asterisk), periportal edema (bold arrows), periductal edema (arrowhead). H-E, x10. Small image. Periductal edema (arrowheads), cholangiocyte damage and degeneration (arrows). H-E, x40

b. Tartrazine group. Necrosis and inflammatory cell infiltration (asterisk) in liver parenchyma. Periportal edema (arrow). H-E, x20

c. Tartrazine group. Apoptotic hepatocytes in liver parenchyma (arrows). H-E, x40

d. Tartrazine group. Liver parenchyma, strong positive caspase-3 immunoreactivity (asterisk), portal region (arrows). H-E, x20

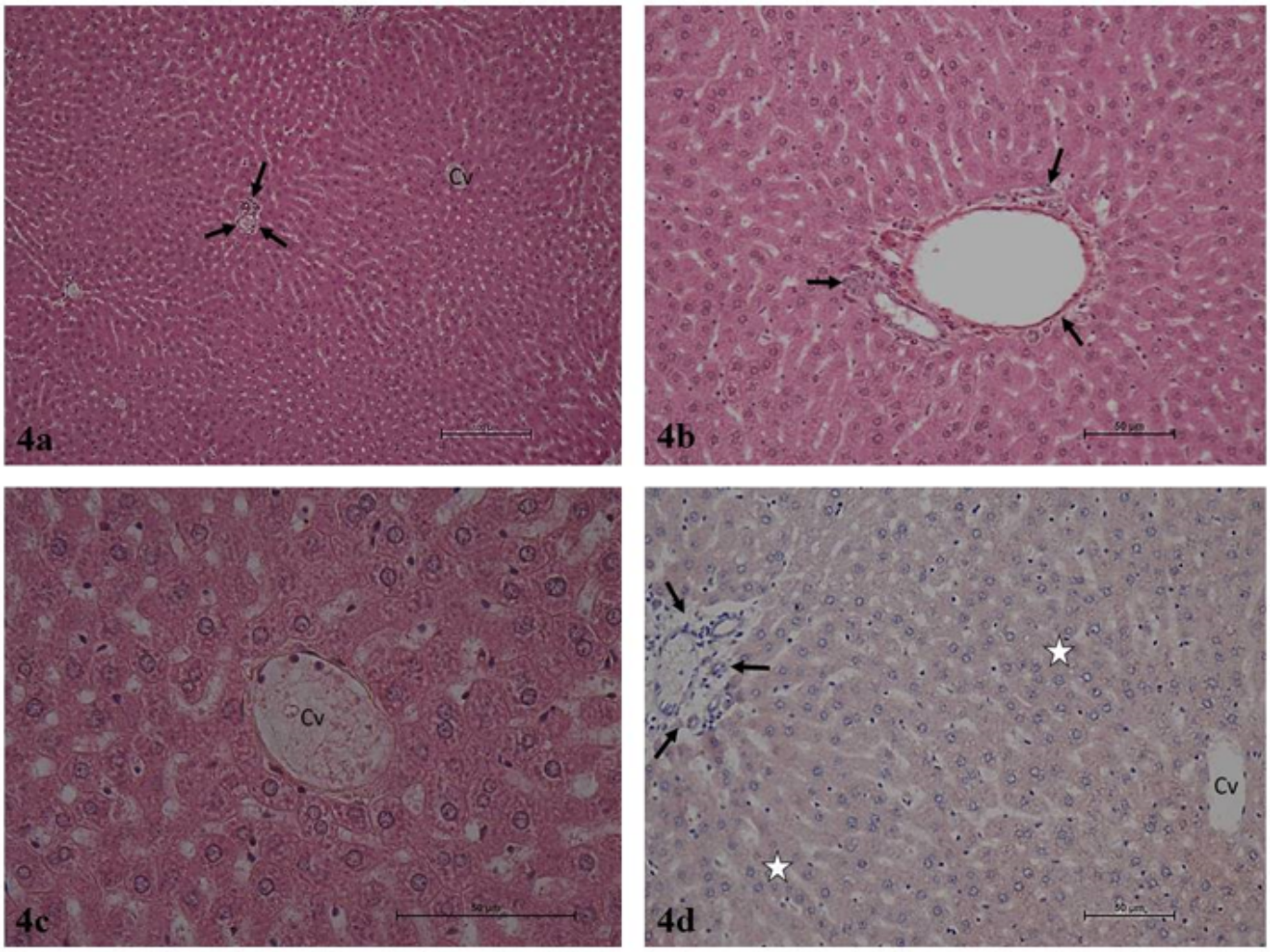

Figure 4

a. Tartrazine + Thymoquinone group. Liver parenchyma in normal histological structure. Central vein (Cv), portal region (arrows). H-E, x10

b. Tartrazine + Thymoquinone group. Liver parenchyma in normal histological structure. Portal region (arrows). H-E, x20

c. Tartrazine + Thymoquinone group. Liver parenchyma in normal histological structure. Central vein (Cv). H-E, x40

d. Tartrazine + Thymoquinone group. Liver parenchyma, weak positive caspase-3 immunoreactivity (asterisk), portal area (arrows). H-E, x20