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Effect of nigella sativa oil on bisphenol a-induced hepatotoxicity in wistar albino rats: histopathological and biochemical investigation

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Abstract

Bisphenol A (or BPA) is a toxic endocrine disruptor that is emitted into the environment as a result of industrial manufacturing methods. In this research, we focused on investigating the protective effects of Nigella sativa oil (NSO) on the liver in rats treated with hepatotoxic BPA. For this purpose, 30 Wistar Albino rats were divided into 4 groups: Control (1 ml olive oil); NSO (5 ml/kg NSO); BPA (100mg/kg); BPA+ NSO (100 mg/kg BPA + 5 ml/kg NSO). All applications were done by oral gavage. At the end of the 30-day study period, blood samples of the anesthetized rats were collected and euthanized under appropriate conditions. After removing the serum of the collected blood samples, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and gamma glutamyl transferase (GGT) levels, which have a key role in liver toxicity, were measured. At the same time, liver samples that were dissected and removed from the cadaver were fixed in 10% formaldehyde solution for histopathological examination and scoring, and hematoxylin - eosin staining were performed. BPA caused degeneration and necrosis in hepatocytes, Kuffper activation, bile duct hyperplasia, congestion, and hepatic cord dissociation, causing serious increases in total liver lesion scores. In parallel, BPA-induced increases were detected in ALT, AST, ALP, and GGT levels. The histological architecture and liver function tests were significantly improved with the addition of NSO to the diet. These findings provided that NSO has a hepatoprotective effect by improving BPA-induced liver damage.

Keywords

BPA, Black Cumin, Hepatotoxicity, Antioxidants, Pathology

Introduction

Xenoestrogens, also known as endocrine-disrupting chemicals (EDCs), are substances that directly affect endocrine functions by acting like hormones produced in the organism or decreasing or increasing their effectiveness. Artificial EDCs, which have been extensively spread in the environment recently, have adverse effects on human and animal health (Michalowicz, 2014; Schug et al., 2011).

Bisphenol A (BPA), one of the most significant of these man-made xenoestrogens, is produced at roughly 6.8 million tons annually (A. Tarafdar et al., 2022). Because BPA is predominantly utilized in manufacturing polycarbonates, epoxy resins, and thermal paper, it may be found in a wide range of everyday items, from water pipes to electrical gadgets, paper, and toys (Hoekstra & Simoneau, 2013; Huang et al., 2012). It is also utilized in materials constantly in contact with food, such as packaging, bottles, and cans (Michalowicz, 2014; Makris et al., 2013). Furthermore, people and animals can be exposed to BPA by inhalation (Geens et al., 2009).

BPA has the potential to interact with specific receptors such as estrogen and androgen receptors, the aryl hydrocarbon receptor connected with different physiological systems, and the peroxisome proliferator-activated receptor (Ziv-Gal et al., 2013). BPA, a lipophilic chemical, can cause various harmful consequences, including oxidative stress, immune system weakness, developmental retardation, reproductive dysfunction, disruption of endocrine homeostasis and genotoxicity (Mukherjee et al., 2020; Murata & Kang, 2018). Furthermore, BPA has been shown to affect the action of many hormones, including sex hormones, leptin, insulin, and thyroxine, as well as induce hepatotoxic,

carcinogenic, mutagenic and immunotoxic consequences (Doherty et al., 2010; Meeker et al., 2010).

It has been reported that BPA causes oxidative stress significantly as a result of decreased activity of antioxidant enzymes and genes and increased free radical production in cells, thus causing protein modifications, lipid peroxidation, and mutations in DNA (Olukole et al., 2019). This results in hepatotoxicity characterized by increased reactive oxygen species and decreased expression of antioxidant genes in hepatocytes (Hassan et al., 2012). The liver is the main organ where many xenobiotics, including BPA, are metabolized, making it a target organ that can be affected even at lower doses than other organs (Diamante et al., 2021; Knaak & Sullivan, 1966). A possible dysfunction in the liver may lead to deterioration of physiological hemostasis with various complications such as increased absorption of toxins in the body, inability to optimize some drugs or treatment regimens metabolized in the liver, digestive disorders, and increased susceptibility to diseases (Bordbar et al., 2021).

The close relationship between healthy nutrition and life expectancy has recently made nutraceuticals very popular in the scientific world (Hatipoğlu & Keskin, 2022a; Hatipoğlu & Keskin, 2022b; İnan et al., 2021; Kısadere, Faruk Aydın, et al., 2021; Kısadere, Karaman, et al., 2021; Gupta & Prakash, 2015). Nigella sativa L. (NS, black cumin), a plant belonging to the Ranunculaceae family, is cultivated in many countries in the eastern Mediterranean, northern Africa, the Indian subcontinent, and Southwest Asia (Hannan et al., 2021). The presence of essential substances such as thymoquinone, thymohydroquinone, thymol, carvacrol, nigellidin, nigellisin, and α-hederin are responsible for its pharmacological and therapeutic benefits in the composition of NS, distinguishes it from other natural compounds and makes it an essential traditional medicine tool (Kooti et al., 2016). Many therapeutic and protective effects of NS, such as anti-analgesic, anti-inflammatory, antioxidant, antiasthmatic, immune-modulating, and hepatoprotective properties, have been reported (Ates & Ortatatli, 2021b; Kooti et al., 2016; Salem, 2005). However, studies examining the protective effects of NS on the negative effects of BPA on the liver are very limited, especially including detailed histopathological analyzes.

As an endocrine disruptor chemical, BPA causes severe damage to many body tissues, organs, and systems, including the liver (A. Tarafdar et al., 2022). Considering the harmful effects of BPA on human and animal life in parallel with its increasing and widespread use in daily life and its bioaccumulation in the body, it appears that it is one of the issues that need urgent attention. Therefore, there is a need for a better understanding of the effects of BPA and new strategies to eliminate or reduce the adverse effects. This study aimed to examine the protective effects of Nigella sativa seed oil (NSO) against the adverse effects of BPA on the liver using enzymes that have a crucial role in evaluating liver functions in histopathology and serum.

Materials and Methods

Chemicals and Other Reagents

BPA was obtained from Sigma Chemical Company (St. Louis, Mo, USA). Nigella sativa seeds oil (NSO) supplied by Botalife (Isparta, Turkey). Alanine transaminase (ALT, Cat. No: DF143), aspartate transaminase (AST, Cat. No: DF41A), alkaline phosphatase (ALP, Cat. No: DC150), and gamma-glutamyl transferase (GGT, Cat. No: DF45A) kits were obtained from Siemens Medical System (Erlangen, Germany). The xylene, alcohol, paraffin, hematoxylin crys., eosin Y solution and Entellan™ used in the histopathological tissue analysis were obtained from Merck Millipore (Darmstadt, Germany). The positively charged slides and coverslips used were purchased from Isolab (Eschau, Germany).

Animals, Ethics Statement and Research Design

Animal experiments were carried out at the Selcuk University Experimental Application and Research Center. The Selcuk University Veterinary Faculty Experimental Animal Production and Research Center Ethics Committee (Approval No: 2022/81) approved the study protocol. All experimental procedures were carried out in accordance with the European Economic Community Directives on animal welfare (86/609/CEE and 2010/63/EU). Thirty male adult Wistar-Albino rats 6-8 weeks old were used in this study. Before beginning the study, the animals' overall health was evaluated. Throughout the study, the rats were housed ad libitum in plastic rat cages in an environment with 12/12 day-night light cycles, room temperature 22±2ºC, and humidity 50±10% percent. The rats, whose body weights were determined, were divided into 4 groups with close mean body weights. After 7 days of acclimatization, they were treated as follows:

1. Control (n=6): 1 ml of olive oil, oral
2. NSO (n=8): 5 ml/kg of N. sativa oil, gavage (Abdel-Zaher et al., 2010).
3. BPA (n=8): 100 mg/kg body weight of BPA (dissolved in 1 ml of olive oil), gavage (Laws et al., 2000).
4. BPA+NSO (n=8): 100 mg/kg body weight of BPA (dissolved in 1 ml of olive oil) + 5 ml/kg of N. sativa oil, orally

The NSO was administered in a dose equivalent to 1.25 per cent of the daily food rate for 30 days. During the trial period, the BPA was prepared fresh each day just before use. In the BPA+ NSO group, NSO was applied 45 minutes after the BPA gavage.

Measurement of Liver Function Test (LFT) Enzyme Activities

To analyze BPA-induced liver damage and NSO's hepatoprotective effects, certain necessary LFT enzymes were measured in the serum. Reitman and Frankel's (1957) standard protocol was used to measure the activity of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) (Reitman & Frankel, 1957). Using the Wenk and Fernandis (2007) protocol, the activities of gamma-glutamyl transferase (GGT) and alkaline phosphatase (ALP) were measured (Wenk & Fernandis, 2007). AST, ALT, ALP, and GGT activity are given in IU/mL of serum.

Histopathological examination

Liver samples were fixed in 10% formaldehyde solution for 24 hours. To get away of the fixative, the tissues were soaked in running water for a full 12 hours. All tissues were embedded in paraffin after regular tissue processing on a tissue processing machine (Leica TP1050). Haematoxylin-eosin (HE) was used to stain sections of paraffin blocks that were cut using a microtome (Leica RM2120). Hepatocyte degeneration

(hydropic - vacuolar and fatty changes), necrosis, bile duct hyperplasia (BDH), hepatic cord dissociation (HCD), congestion, Kupffer cell activation (KCA), karyomegaly, and mononuclear cell infiltration (MCI) were examined under a microscope in at least five different regions. The severity and prevalence of these results were scored as follows: (0): no lesion; (1): 1–25 percent; (2): 26–50 percent; (3): 51–75 percent; and (4): 76–100 percent. Following the collection of the numerical values assigned to the degenerative findings and the total lesion score of that replicate was calculated (maximum score is 28). An expert who was not aware of the experimental groups performed the histopathological inspection and scoring.

Statistics analysis

Normal distribution analyzes of liver function tests and histopathological scoring were done with the Kolmogorov-Smirnov test. The homogeneity of variances was controlled using Levene's test. All data were evaluated by the Duncan analysis following one-way ANOVA (SPSS® program). Statistical importance was described as a value of ($p<0.05$).

Results and Discussion

Evaluation of the effects of 30-day BPA application on LFT enzymes is presented in Figure 1. BPA caused a statistically significant increase in AST, ALT, ALP and GGT activities in the serum of rats compared to the control group ($p<0.05$). This increase in the BPA group compared to the control group was 41.04% for AST, 25.13% for ALT, 58.15% for ALP and 97.70% for GGT. NSO applied simultaneously with BPA showed significant decreases in LFT enzyme levels compared to the BPA group ($p<0.05$). Compared to the BPA group, this decrease in BPA+NSO was 22.37% for AST, 42.46% for ALT, 18.88% for ALP, and 28.24% for GGT. The difference between the control and NSO groups for all enzymes was not significant ($p>0.05$) (Figure 1).

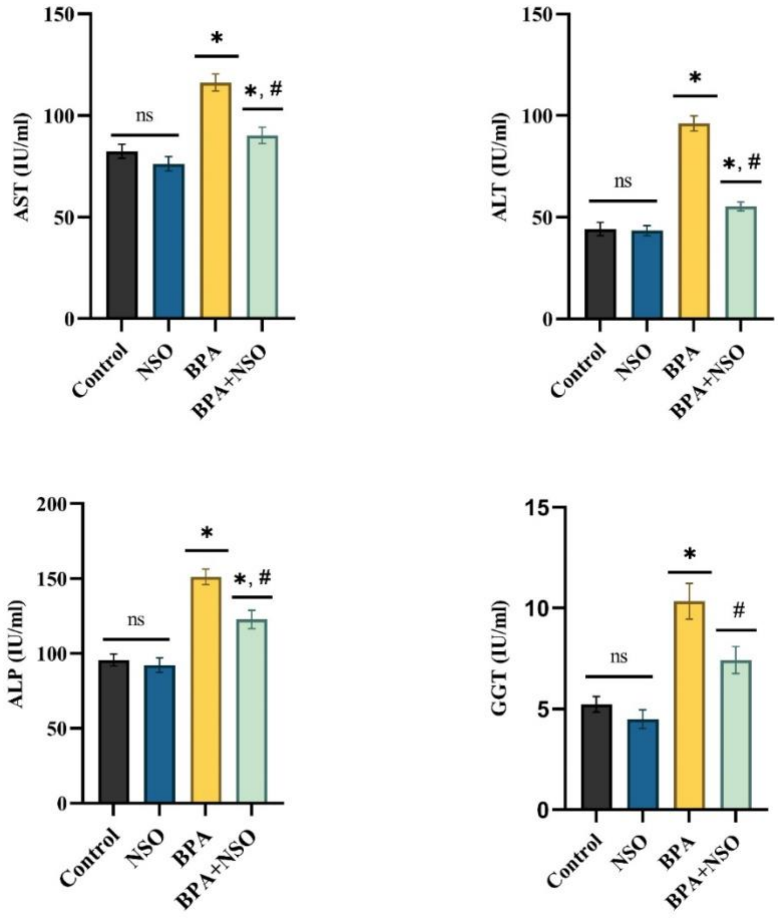

Figure 1. Effect of BPA and NSO on Serum Liver Function Test (LFT) Enzymes. Aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP) and gamma glutamyl transferase (GGT) values are expressed as IU/mL of rat serum samples. *; significant differences compared to the control group ($p<0.05$), #; significant differences according to BPA group ($p<0.05$), ns; shows that the difference between groups is insignificant ($p>0.05$).

Histopathological examination was performed for each case for hepatocyte degeneration (hydropic-vacuolar and fatty changes), necrosis, bile duct hyperplasia (BDH), hepatic cord dissociation (HCD), congestion, Kupffer cell activation (KCA), karyomegaly, and mononuclear cell infiltration. Afterwards, the findings were scored separately and the total lesion score was determined (Table 1, Figure 2). When the results were examined, it was observed that degenerative changes and necrosis in hepatocytes, BDH, HCD, congestion, and KCA were significantly increased in the BPA group ($p<0.05$). The addition of NSO to the diet, along with BPA, was found to significantly normalise these conditions. There was no significant difference between BPA group and other groups in terms of karyomegaly and MCI ($p>0.05$). Although degenerative and necrotic changes in

hepatocytes condensed in the centrilobular region, it was observed that they were also present in other regions. Although the total lesion score obtained from all these findings showed a very serious increase in the BPA group, this increase was prevented by the addition of NSO ($p<0.01$). In terms of the parameters examined, no difference was found in the groups given only NSO compared to the healthy control group ($p>0.05$).

Table 1. Histopathological results

	Hydropic Degeneration/ Fatty changes	Necrosis	BDH	HCD	Congestion	KCA	Karyomegaly	MCI	Total lesion score
Control	0.75±0.17[a]	0.50±0.18[a]	0.50±0.18[a]	0.58±0.08[a]	0.33±0.11[a]	0.50±0.00[a]	0.50±0.00	0.58±0.08	4.33±0.57[a]
NSO	0.67±0.11[a]	1.00±0.18[a]	0.50±0.00[a]	0.50±0.00[a]	0.83±0.28[a]	0.50±00[a]	0.50±0.00	0.42±0.08	4.92±0.57[a]
BPA	1.25±0.21[b]	2.67±0.25[b]	2.75±0.21[b]	1.50±0.18[b]	2.67±0.40[b]	0.83±0.17[b]	0.58±0.08	0.58±0.08	12.83±1.46[b]
BPA+ NSO	0.58±0.08[a]	0.50±0.00[a]	0.58±0.08[a]	0.50±0.00[a]	0.50±0.00[a]	0.50±0.00[a]	0.50±0.00	0.50±0.00	4.17±0.26[a]
	p<0.05	p<0.01	p<0.01	p<0.01	p<0.01	p<0.05	p>0.05	p>0.05	p<0.01

[a,b] Values with different superscripts in the same column indicate that the difference is statistically significant ($p<0.05$, one-way ANOVA post hoc Duncan test). BDH: Bile duct hyperplasia; HCD: Hepatic cord dissociation; KCA: Kupffer cell activation; MCI: mononuclear cell infiltration.

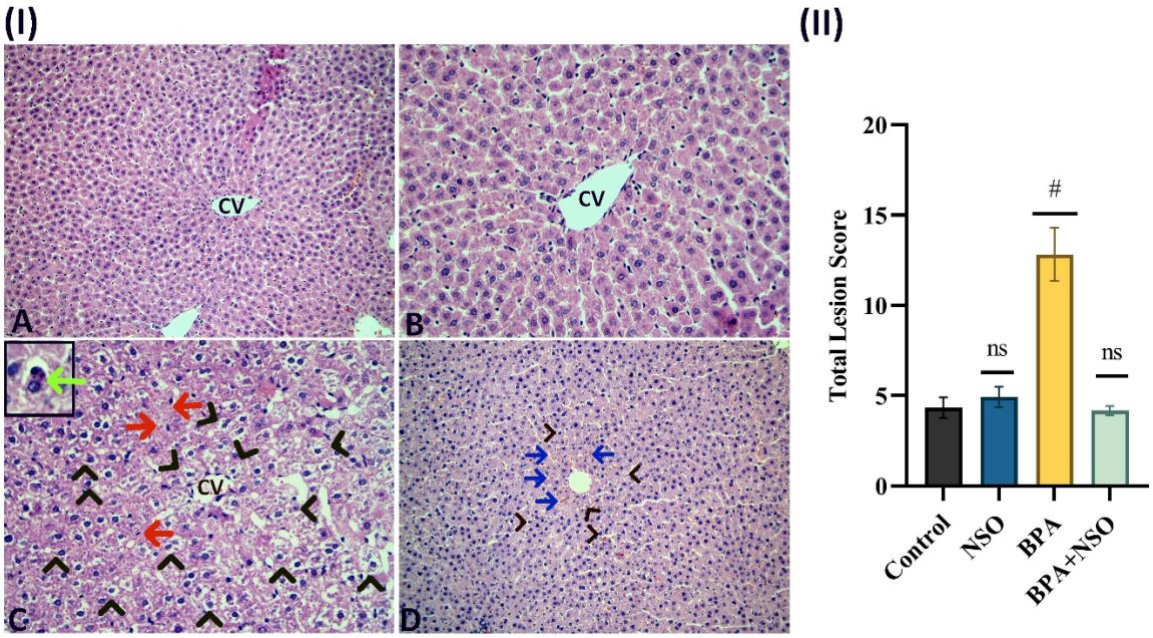

Figure 2. (I) Photomicrographs of liver, A: Control, 10X, HE; B: NSO group, 40X, HE; C: BPA group, 20X (Inset: 40X), HE; D: BPA+NSO group, 10X, HE. CV: Central vein, Green arrow: Apoptosis of hepatocytes, Arrow heads: Dejeneration of hepatocytes, Red arrows: Necrosis of hepatocytes, Blue arrows: Congestion. (II). Graphical representation of liver total lesion score. #; the statistical difference compared to the control group was significant ($p<0.01$), ns; shows that the difference between the groups is insignificant compared to the control group ($p>0.05$).

Studies on the toxic effects of BPA, an endocrine disrupting chemical that spreads at alarm level to the environment, mostly focused on the reproductive system, and studies investigating its effects on the liver were found to be very limited (Ayon Tarafdar et al., 2022). Therefore, the present study aimed to eveluate the potential of NSO to prevent BPA-induced liver injury in rats. For this purpose, the protective effect of NSO administered at a dose of 5 mg/kg against structural and cellular damage caused by BPA in the liver, as well as the changes in LFT enzyme levels observed as a result of BPA exposure were examined.

BPA is largely absorbed through the skin, causing damage to the kidneys and liver (Uzunhisarcikli & Aslanturk, 2019). Since the liver is the organ primarily responsible for BPA metabolism, it has been reported to be more sensitive to even low doses compared to other organs (Diamante et al., 2021; Knaak & Sullivan, 1966). The biochemical results of the current study clearly demonstrated the increase in serum ALT, AST, ALP and GGT activities in BPA-treated rats (Figure 1). LFT, including ALT, AST, ALP, GGT enzyme levels, and histopathological examination of liver tissue have a key role in determining hepatotoxicity and are accepted as widely used examination tools (Tian et al., 2019). ALT and AST are two important enzymes found in high concentrations in the liver (Goorden et al., 2013). Serum AST and ALT levels are constantly measured in routine laboratory examinations as indispensable parameters used in the detection of liver damage (Center, 2007). Although most transaminases are located in the soluble fraction of the cytosol, a significant portion of AST is found in the mitochondria (Moshtaghie et al., 2003). Transaminases are distributed differently within the acinar regions. ALT concentrations are higher in periportal hepatocytes, while AST are higher in periacinar (zone 3) hepatocytes. In conclusion, the activation of ALT and AST in serum may indicate that liver injury is more concentrated in the acinar and periportal region (Rej, 1989). ALP, a critical biomarker for the detection of hepatobiliary disorders, is found in a variety of tissues including bone, liver, intestine, placenta, and other organs (Sharma et al., 2014).

A high level of ALP has also been linked to extrahepatic biliary obstruction, intrahepatic mass pathologies, rickets, and malignancy (Zhang et al., 2021). In toxicological studies, measurement of ALP activity is frequently used in the diagnosis of damage to different tissues as a result of exposure to toxic substances (Nangia & Yadav, 2021). GGT is located in the plasma membrane of hepatocytes and its elevated activity is widely recognized to be an indication of liver injury (Whitfield, 2001). Long-term exposure to BPA can cause histopathological changes characterized by degeneration and/or necrosis of hepatocyte, which is reflected by abnormally increased serum AST, ALT, ALP and GGT levels (Sun et al., 2021; Meng et al., 2019). Indeed, Korkmaz et al. (2010) reported that they detected necrosis and congestion and an increase in AST and ALT levels in the BPA-treated rats, and the reason for this was related to BPA-induced peroxidation of membrane lipids in hepatocytes (Korkmaz et al., 2010). Al-Seeni et al. (2016) reported that NSO improved ALT, AST and ALP levels against CCl4-induced hepatotoxicity (Al-Seeni et al., 2016), while Hamza and Al-Harbi (2015) reported that NSO treated LFT enzyme activities against paracetamol-induced liver damage (Hamza & Al-Harbi, 2015). In the current study, the improvement of abnormally increased ALT, AST, ALP and GGT enzyme levels when NSO administered simultaneously with BPA was evaluated as a remarkable finding that was interpreted as NSO reduced BPA-induced liver damage.

The devastating toxic effects of BPA on the liver have been demonstrated by researchers (Han & Hong, 2016). The most important reason for the harmful cellular effect of BPA in the liver is attributed to increased oxidative damage (Bindhumol et al., 2003). BPA not only causes the accumulation of reactive oxygen species in hepatocytes, but also causes hepatotoxicity by decreasing the expression of antioxidant genes and enzymes (Hassan et al., 2012). Increased ROS production has been shown to cause degeneration and necrosis/apoptosis in hepatic stellate cells, Kupffer cells (KC), and hepatocytes by interfering with mitochondrial energy metabolism (Iwakiri, 2015). Based on this evidence, it was thought that the main reason for the degeneration and necrosis, which was revealed histopathologically in the current study and concentrated especially in the centrilobular region, was the increased cellular ROS and decreased antioxidant gene and enzyme expression induced by BPA.

When Kupffer cells, hepatic macrophages localized in the lumen of liver sinusoids, are activated, they can cause the secretion of certain cytokines that play a role in the pathophysiology of liver diseases (Bordbar et al., 2021). Similarly, duct hyperplasia and congestion can be observed even due to hepatotoxicity (Greaves, 2007). Oral administration of BPA has been reported to cause liver damage, characterized by KC activation, degeneration and necrosis of hepatocytes (Bordbar et al., 2021; Mourad & Khadrawy, 2012). In the current study, in parallel with other studies, it was demonstrated histopathologically that BPA causes hepatocyte degenaration and necrosis as well as KC activation, bile duct hyperplasia, congestion, and increases the total liver lesion score.

The current study's most crucial starting point was determining NSO's protective actions against BPA-induced hepatotoxicity. NSO was found to provide exciting and significant improvements in hepatocyte degeneration and necrosis, KC activation, bile duct hyperplasia, congestion, and, finally, total liver lesion score. Although studies investigating the combined effects of BPA and NSO on the liver are very limited, it was thought that this effect of NSO might be due to its strong antioxidant effects, based on some studies with other antioxidant natural compounds (Abdel Samie et al., 2018; Bordbar et al., 2021; Kazmi et al., 2018; Kooti et al., 2016; Olukole et al., 2019; Zaulet et al., 2017). It has also been highlighted that NS and its pharmacologically active substance, thymoquinone, have hepatoprotective effects by inhibiting nuclear receptors responsible for xenobiotic biotransformation in the liver and increasing the expression of glutathione and glutathione S-transferase alpha 3, which are responsible for increasing antioxidant capacity (Ates et al., 2022; Ates & Ortatatli, 2021a). In addition, the fact that the parameters examined in the group given only NSO were not different from the healthy control group was accepted as an indication that NSO did not have a toxic effect at the doses used in the study.

Conclusion

It has been determined that BPA administration causes complications characterized by degeneration and necrosis, Kuffper cell activation, congestion, bile duct hyperplasia and dissociation in hepatocytes, and this structural and cellular damage in the liver results in an increase in serum liver enzymes. Significant findings have been obtained that the simultaneous addition of NSO to the diet has a hepatoprotective effect by reducing the aforementioned BPA-induced liver damage and contributes to the provision of physiological hemostasis.

Compliance with Ethical Standards

Conflict of interest

The authors declared that for this research article, they have no actual, potential or perceived conflict of interest.

Author contribution

Designed of study: MBA, DH; Pathological analyses: MBA; Blood analyses: DH; Writing the Article: MBA, DH; Critical Review: MBA. All the authors read and approved the final manuscript. All the authors verify that the Text, Figures, and Tables are original and that they have not been published before.

Ethical approval

The Selcuk University Veterinary Faculty Experimental Animal Production and Research Center Ethics Committee (SUVDAMEK), Approval No: 2022/81

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Data availability

Not applicable.

Consent for publication
Not applicable.

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