Journal of Animal & Plant Sciences, 35(1): 2025, Page: 90-105
ISSN (print): 1018-7081; ISSN (online): 2309-8694
https://doi.org/10.36899/JAPS.2025.1.0007

FUCOIDAN FROM *Fucus vesiculosus*, REGULATES OXIDATIVE AND TRANSCRIPTIONAL RESPONSES IN THE SULFOXAFLOR EXPOSED MICE LIVER: ASSESMENT OF DNA DAMAGE GENES, THAT REPAIR DNA DAMAGE (XRCC1, OGG1, APE1, AND PARP1), AND THE ANTIOXIDANT STATUS

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ABSTRACT

This research aimed to determine regulatory role of sulfated polysaccharides fucoidan from *Fucus vesiculosus* against oxidative and transcriptional responses in sulfoxaflor exposed mice liver. For this purpose both sulfoxaflor and fucoidan were given orally to mice for 24 hours and 7 days at doses of 15 mg/kg/day (equivalent to 1/50 oral LD_{50}) and 50 mg/kg/day. At the end of the tests, liver samples were collected and used to assess 8-OHdG levels, the mRNA expression levels of DNA damage response genes such as XRCC1, OGG1, APE1, and PARP1. Furthermore, levels of tGSH and enzyme activity of GPx, GR, and GST, as well as TBARS, were also examined. The current study's findings demonstrated that acute sublethal exposure to sulfoxaflor caused lipid and DNA damage in mice liver via raising TBARS and 8-OHdG levels, respectively, and activating antioxidants linked to GSH. Furthermore, sulfoxaflor increased the mRNA expression of XRCC1 and APE1 genes, which are involved in the DNA repair mechanism. This tudy indicated that sulfoxaflor caused oxidative responses via increasing 8-OHdG and TBARS levels and altering the antioxidant status. Fucoidan protected liver cells from sulfoxaflor-induced oxidative effects and regulated the DNA damage response at the transcriptional level in mice liver.

Keywords: Fucoidan, Sulfoxaflor, DNA damage, DNA repair genes, antioxidant status

Published first online December 20, 2024 Published final February 18, 2025

INTRODUCTION

Neonicotinoids are potent neuroactive insecticides due to their neurotoxic effects on nicotinic acetylcholine receptors (nAChRs) (Costas-Ferreira and Faro, 2021). Although neonicotinoids are classified as a relatively new class of insecticide among the five main chemical insecticide classes, their use has increased globally in the last two decades and constituted 26% of the global market (Tomizawa, 2005; Jeschke and Nauen, 2013; Casida and Durkin, 2013). Neonicotinoids are predominantly used in Latin America, Asia, and North America, accounting for 75% of total global sales (Bass *et al*., 2015). Compared to many other insecticides, neonicotinoids have different physico-chemical characteristics. They have low organic-carbon partitioning coefficients (KOC, 26 to 601 mL/g) and high water solubility (184 mg/L to 590,000 mg/L at 20 °C and pH 7) which leads to high leaching and runoff potentials (Morrissey *et al*., 2015). Their water solubility enables neonicotinoids to infiltrate ground and surface water systems. Due to their stability and low soil binding, they present a substantial risk of water source contamination and can persist in soil for extended periods-often months to years—after application (Aseperi *et al*., 2020; Stehle *et al*., 2023). Recent studies indicate that neonicotinoids' environmental persistence can lead to prolonged exposure of non-target animals and humans through food, water, and even inhalation of dust particles (Wood and Goulson, 2017). Neonicotinoids have been detected across various biological and environmental matrices, including sediment, soil, dust, water, pollen, honey, nectar, and fruits (Bonmatin *et al*. 2015).

Sulfoxaflor (CAS: 946578-00-3) ([methyl(oxo)1-[6-(trifluoromethyl)-3-pyridyl]ethyl-6-sulfanylidene] cyanamide) is a neonicotinoid-like pesticide functioning as an agonist of nAChRs (Watson *et al*., 2021). This systemic insecticide is effective against sap-feeding insect pests, such as aphids, whiteflies,

hoppers, and *Lygus* (Zhu *et al*., 2011). Sulfoxaflor has been proposed as an alternative to the use of neonicotinoids and its use has been increasing worldwide since its approval in US and EU (Bass *et al*., 2015). The potential bioaccumulation of sulfoxaflor along the food chain could elevate risks to non-target species (Xu *et al*., 2020).

Research has extensively documented the neurotoxic potential of neonicotinoids in mammals (Rodrigues *et al*., 2010), including their ability to induce oxidative stress (El-Gendy *et al*., 2010; Zhang *et al*., 2011; Mohany *et al*., 2011; Aydin, 2011; Kapoor *et al*., 2011; Duzguner and Erdogan, 2010). Emerging evidence suggests that sulfoxaflor also induces oxidative stress in rats, impacts reproductive systems (Mohamed *et al*., 2022), and may exhibit apoptotic, cytotoxic, and genotoxic effects in human lymphocyte cell cultures (Sınacı *et al*., 2022). Moreover, sulfoxaflor has shown carcinogenic (LeBaron *et al*., 2013; Rasoulpour *et al*., 2014) and teratogenic properties (Rasoulpour *et al*., 2012) in mammals, with findings indicating carcinoma and adenoma formation in liver tissue in both rats and mice (LeBaron *et al*., 2013).

Reactive oxygen species (ROS) compromise cellular integrity by altering proteins, membrane lipids, nucleic acids, and the extracellular matrix, resulting in oxidative stress and cellular damage (Juan *et al*., 2021). Lipid peroxidation, a frequently studied process linked to ROS-induced damage, underscores ROS's impact on cellular structures (Su *et al*., 2019). ROS from exogenous sources, such as ionizing radiation, UV light, carcinogens, pollutants, and metabolic processes, can also induce DNA damage (Wolters and Schumacher, 2013; Aranda-Rivera *et al*., 2022). ROS-mediated DNA lesions encompass strand breaks and nucleotide base modifications. Cells deploy repair mechanisms such as base excision repair (BER), homologous recombination, and non-homologous end joining to mitigate DNA damage (Helena *et al*., 2018). ROS causes some modifications in DNA, such as base and sugar lesions, single-strand breaks, abasic regions, and DNA-protein cross-linking. The most frequently occurring oxidative base damage product of ROS in DNA and the best-known mutagen is 8-hydroxy-2'-deoxyguanosine (8-OHdG). ROS-induced oxidative damage to DNA is one of the leading causes of diseases resulting from impaired tissue activities, including cancer, cardiovascular disease, immune system diseases, and degenerative diseases. 8-OHdG is accepted as an indicator of DNA oxidative damage and is the most frequently used marker to determine oxidative DNA damage (Valavanidis *et al*., 2009). Several pesticide groups have been associated with elevated 8-OHdG levels in both *in vitro* and *in vivo* studies (Rumsey *et al*., 2017; Zhou *et al*., 2018).

Although there are many DNA repair mechanisms in cells, considering the potential of ROS to cause damage to DNA, the base excision repair (BER) mechanism, which is involved in the repair of single-strand breaks and nucleotide base exchange, is critical for such damage (Jalal *et al*., 2011, Kaur *et al*., 2018). Proteins involved in DNA repair are considered fundamental responses to oxidative stress-induced DNA damage. Notably, pesticide-induced oxidative stress can lead to dysregulation at the transcriptional level of genes encoding key DNA repair proteins (PARP1, OGG1, APE1, and XRCC1), potentially contributing to diseases like cancer (Thakur *et al*., 2018). While specific studies have elucidated the toxic effects of sulfoxaflor, its effect on the expression levels of genes involved in oxidative stress-induced DNA damage and repair mechanisms has yet to be reported. Considering that the disruption of gene expression level regulation is directly related to many diseases (Wu *et al*., 2019), determining the effect of sulfoxaflor on this signaling pathway at the transcriptional level is critical.

Each cell possesses an antioxidant defense mechanism to scavenge ROS and mitigate oxidative stress-related cellular damage. Among the non-enzymatic antioxidants, the most important is glutathione (GSH) (Pizzino *et al*., 2017). GSH has numerous cellular functions, including non-enzymatic or enzymatic detoxification of electrophilic xenobiotics through the glutathione S-transferase (GST; EC 2.5.1.18) family. GSTs participate to the defense against oxidative stress by detoxifying endogenous harmful substances such as hydroxyalkenals, base propenals (byproducts of lipid peroxidation), and DNA hydroperoxides (Cnubben *et al*., 2001). Additionally, GSH aids in removing ROS from the cell through the catalytic activities of glutathione peroxidase (GPx; EC 1.11.1.9) and glutathione reductase (GR; EC 1.8.1.7) (Forman *et al*., 2009). Recently, compounds with pharmacological potential sourced from natural environments have drawn researchers' interest (Atanasov *et al*., 2021). Fucoidan, a sulfated polysaccharide derived from macroalgae, has attracted attention due to its diverse therapeutic properties, including antioxidant, anti-inflammatory, and hepatoprotective effects.

Fucoidan, a sulfated polysaccharide obtained from macroalgae species, exhibits diverse biological activities and therapeutic properties, including anticancer (Aisa *et al*., 2005, Nagamine *et al*., 2009, Han *et al*., 2015) anti-viral (Li *et al*., 1995, Schaeffer and Krylov, 2000, Mandal *et al*., 2007, Doh-Ura *et al*., 2007) anti-coagulant (Nishino *et al*., 1991; Dobashi *et al*., 1989, Chandia and Matsuhiro, 2008), anti-inflammatory (Maruyama *et al*., 2005) and antioxidant effects (Sanjeewa *et al*., 2018). Recent reports demonstrated that fucoidan is a potential ROS scavenger and essential for free-radical, thus playing an important role in protecting cells, tissues, and organs from disturbing effects of toxicants and diseases by preventing oxidative damage

(Chandini *et al*., 2008; Sithranga Boopathy and Kathiresan, 2010; Hong *et al*., 2012, Lim *et al*., 2015; Wang *et al*., 2024). Fucoidan has attracted considerable attention due to its potential therapeutic applications, particularly in the context of liver health. Numerous studies have indicated its hepatoprotective properties against various liver injuries (Dimitrova-Shumkovska *et al*., 2020). The exact processes responsible for the liver protective actions of fucoidan are complicated and varied, although several pathways have been identified: antioxidant activity (Dimitrova-Shumkovska *et al*., 2020), anti-inflammatory effects (Wang *et al*., 2024), regulation of fibrosis (Hayashi *et al*., 2008) antiviral activity against hepatitis B virüs (Li *et al*., 2017) enhancement of liver regeneration (Wang, *et al*. 2018).

We have previously shown that sulfoxaflor produced oxidative stress and altered GSH-related antioxidant systems in zebrafish (*Danio rerio*) gills (Piner Benli and Celik, 2021a). Additionally, as an antioxidant, fucoidan may protect mice from oxidative stress and sulfoxaflor-induced hematological/biochemical changes (Piner Benli *et al*., 2021b) as well as modulate caspase-3 mRNA expression and oxidative stress in the brains of mice exposed to sulfoxaflor (Piner Benli *et al*., 2021c). The current study aims to investigate the oxidative stress-induced DNA damage potential of sulfoxaflor in the liver of male Swiss albino mice (Mus musculus) and its effects on DNA repair mechanism at the transcriptional level, as well as the possible protective and regulatory role of fucoidan.

MATERIALS AND METHODS

Chemicals: Fucoidan (*Fucus vesiculosus*, Sigma F5631) was purchased as a powder from Sigma-Aldrich Co. Sulfoxaflor (CAS number: 946578-00-3), ([methyl (oxo) {1- [6- (trifluoromethyl) -3-pyridyl] ethyl} -λ6-sulfanylidene] cyanamide) were purchased as a commercial product (Transform) 500WG (%50 w/w active ingredient) from a distributor company. All other chemicals of analytical grade and kits for biochemical and molecular analysis were obtained from Sigma-Aldrich, Merck, Elabscience, Promega, M-N and NEB.

Animals: The Experimental Medicine Research and Application Centre (Türkiye) of the Faculty of Medicine provided the 26 g ± 2 g Swiss albino mice (*Mus musculus*). The test animals were housed at 20±2°C temperature, 50-60% humidity, 12-h light–dark cycle, and kept to acclimatize for two weeks before the experiments. They were fed standard pellet form food (TAVAŞ®) and provided with water ad libitum. The Experimental Medicine Research and Application Center Ethics Committee of the Faculty of Medicine approved the experimental protocol (Approval Code: 9, Approval Date: 04.11.2019) at Cukurova University. International guidelines for the care and use of laboratory animals are conducted in all experimental procedures.

Experimental Procedure: Eight groups including eight mice each were randomly selected from a total of sixty-four male mice. Acute oral toxicity tests (24-h, 7-day) complied with OECD guidelines (OECD, 2021). The first (control) group received ad physiological saline from oral gavage (24 h). The second group (fucoidan) was given a single oral dose of fucoidan in physiological saline (50 mg/kg bw) daily by oral gavage (24 h). In comparison, the third group (sulfoxaflor) received a single oral dose of sulfoxaflor in physiological saline (15 mg/kg bw) daily by oral gavage (24 h). Animals in the fourth group (sulfoxaflor+fucoidan) were given a single oral dose of sulfoxaflor (15 mg/kg bw) per day, two h after the onset of fucoidan (50 mg/kg bw) administration (24 h). The other four toxicity test groups were set similarly to four treatment groups for 7-day treatment periods. The dose of sulfoxaflor used in this study represented 1/50 of oral LD_{50} (750 mg/kg bw) (Brooks *et al*., 2008, Piner Benli *et al*., 2021b) Fucoidan was administered to mice orally at 50 mg/kg/day, according to previous studies (Li *et al*., 2002, Ponnan *et al*., 2020)

At the end of the experiment, the animals in different groups were euthanized by ketamine/xylazine and cervical dislocation for liver sample collection. The liver was dissected out, weighed, and washed using phosphate buffer on an ice plate. All the tissue samples were carried in a container filled with liquid nitrogen, and then stored at a temperature of -80°C.

Biochemical Assays

Determination of Antioxidant Parameters: Liver samples were homogenized with a 0.05 M Na-P buffer (pH 7.4) containing 0.25 M sucrose at 1/10 (w/v) with a homogenizer (Qiagen Tissue Ruptor II, Hilden, Germany) at 10000 rpm for 5 minutes, and the homogenates were centrifuged at 10000*xg* for 30 minutes at +4°C (Hettich Micro 220, Tuttlingen, Germany). Liver homogenates were mixed with 10% sulfosalicylic acid at a ratio of 1:0.5 (v/v). The mixture was then centrifuged at 10000*xg* for 5 minutes to determine the tGSH levels. A UV-Vis spectrophotometer (Shimadzu UV-Vis Spectrophotometer UV-1700) was used to quantify the levels of tGSH and the activity of the enzymes GPx, GR, and GST. The method developed by Anderson was used for the determination of tGSH (Anderson, 1985). The tGSH level was quantified by a recycling reaction involving 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB) and GR. The reaction medium contained 143 mM sodium-potassium buffer (6.3 mM EDTA, pH 7.5), 0.3 mM NADPH, 6 mM DTNB, and 50 units of glutathione reductase GR. Then, the absorbance was measured at 412 nm and 30 °C. The results were converted to concentrations utilizing a standard graph prepared with

GSH. The GPx-specific activity was determined at 340 nm at 37ºC by measuring NADPH consumption by GR using t-butyl hydroperoxide as a substrate (Beutler, 1984). NADPH oxidation was measured by GSSG at 37 °C and 340 nm to assess the GR-specific activity (Carlberg *et al*., 1975). 1-chloro-2,4-dinitrobenzene (CDNB) was used as a substrate to assess the activity of GST. The rate of conjugation of GSH (20 mM) with CDNB (1 mM) was measured (Habig *et al*., 1974).

Determination of Oxidative DNA Damage (8-OHdG): 8-OHdG levels in liver samples were determined using the Competitive ELISA method according to the protocol in the commercially available 8-OHdG ELISA Kit ((Elabscience, E-EL-0028, www.elabscience.com) with Microplate Reader (Biotek ELx800, Biotek Intruments, Inc., Winooski, VT, USA). Liver samples were homogenized with a glass homogenizer using 1/10 (w/v) phosphate buffer (0.01M, pH=7.4), and the freeze-thawed process was applied, then centrifuged at 5000×g for 5 minutes.

Lipid Peroxidation Assay: The level of lipid peroxidation was quantified with a UV-Vis spectrophotometer (Shimadzu UV-Vis Spectrophotometer UV-1700) using the thiobarbituric acid reactive substances (TBARS) assay (Wills, 1966). Using a standard curve created with 1,1,3,3-tetraethoxypropane, TBARS levels were determined.

Protein Determination: Bradford's method was used to determine the protein concentration in liver homogenates using Bradford Reagent with UV-Vis spectrophotometer (Shimadzu UV-Vis Spectrophotometer UV-1700) (Bradford, 1976).

Molecular Assays

Real-time PCR for Determination of Target Genes Expression: A commercial kit extracted Total RNA from liver samples (Nucleozol, MN, Duren, Germany). The RNA concentrations were determined as 0.5–2.5 µg/µl by Qubit 2.0 fluorometer (Life Technologies, Carlsbad, CA, USA). In the next step, cDNA samples were obtained using the isolated RNA samples with the help of a synthesis kit (Protoscript II First Strand cDNA synthesis kit, NEB, Ipswich, MA, USA). XRCC1, OGG1, APE1, and PARP1 transcriptional levels were determined via RT-PCR utilizing synthesized cDNA and specific primers: XRCC1-F: CGTATGCAGGCTCCACAGA and XRCC1-R: GTTTTCCATCAGGGCCTCC, OGG1-F: TGCTGGCAGATCAAGTATGG and OGG1-R: CAGCAGTCTCACACCTTGGA, APE1-F: CTAAGGGCTTTCGTCACAGC and APE1-R: GCCTCCTTCTCGGTTTTCTT, PARP-F: CTCTCCCAGAACAAGGACGAAG and PARP-R: CCGCTTTCACTTCCTCCATCTTC. All primers were designed according to the criteria outlined by Raymaekers *et al*. (2009), while the PARP primers were selected from Honarpisheh *et al*. (2016). A reaction mix including 1 µl of cDNA sample, 0.5 µl of each specific primer (10 µM), 10 µl GoTaq qPCR Master Mix (Promega, Madison, WI, USA), and 8 µl RNase-free water was made in a total of 20 ul. The process for thermal cycling was as follows: one cycle of 95 °C for 2 min, 40 cycles of 95 °C for 15 s, and 60 °C for 60 s). Amplifications were performed in an ABI 7500 (Applied Biosystems, Singapore). GAPDH (GAPDH-F: CCAGCTACTCGCGGCTTTA; GAPDH-R: ACGGCCAAATCCGTTCACA) (Raymaekers *et al*., 2009) was used as the housekeeping gene in data normalization. The gene expression levels for each group were assessed utilizing the $2^{-\Delta\Delta Ct}$ methodology (Livak and Schmittgen, 2001). All procedures were executed following the manufacturer's provided instructions.

Statistical Analysis: Statistical comparisons between the control and test groups were conducted using one-way ANOVA, followed by Duncan's multiple comparison test for biochemical parameters and Tukey's test for molecular parameters in SPSS 22.0. All data are presented as mean values with standard error

RESULTS

Effects of Sulfoxaflor and Fucoidan on the Antioxidant System: Tables 1 and 2 present the effects of sulfoxaflor and fucoidan on the GSH-dependent antioxidant system. Results indicated a significant increase in tGSH levels in the sulfoxaflor group after 24 hours compared to the control. However, tGSH levels decreased significantly in both the sulfoxaflor and sulfoxaflor+fucoidan groups after 7 days compared to the control ($p \leq 0.05$). At the 7-day mark, the reduction in tGSH levels in the sulfoxaflor+fucoidan group was not statistically significant compared to the sulfoxaflor group ($p \leq 0.05$).

Table 1. Changes in tGSH levels in mice liver (µM/mg protein)

	tGSH			
	Control	**Fucoidan**	**Sulfoxaflor**	**Sulfoxaflor+Fucoidan**
24h	7.14 ± 0.14^{bx}	7.52 ± 0.37^{abx}	8.29 ± 0.38^{ax}	$6.90\pm0.36^{bx*}$
7d	7.29 ± 0.20^{ax}	6.87 ± 0.34^{ax}	5.63 ± 0.22^{by}	5.11 ± 0.46^{bx}

Values are expressed as mean ±standard error. Mean values bearing different superscripts in a row differ significantly ($P \leq 0.05$)

The differences between groups at the same duration are represented by the letters a and b the differences between groups at different durations are represented by the letters x and y. At the P≤0.05 level, the data with different letters are statistically different (n = 8). Asterisks (*) show the differences between sulfoxaflor groups and sulfoxaflor+fucoidan groups.

GPx enzyme activity in the liver was significantly elevated compared to the control group following 24-day exposures to fucoidan. Sulfoxaflor increased GPx activity at the end of the 7 days. Notably, the decrease in GPx activity in the combined sulfoxaflor+fucoidan treatment group was statistically significant relative to the sulfoxaflor-only group at the end of 7 days ($p \leq 0.05$). After 24 hours, GR enzyme activity remained unchanged in all groups; however, a significant increase in GR activity was observed in the sulfoxaflor, fucoidan and sufoxaflor+fucoidan groups at the 7-day mark ($p \leq 0.05$). GST enzyme activity showed a reduction across all treatment groups at the end of 24 hours, with the most pronounced decrease noted in the sulfoxaflor+fucoidan group. By the 7-day endpoint, GST activity had decreased significantly in both the fucoidan and sulfoxaflor+fucoidan groups ($p \leq 0.05$).

Table 2. Changes in GPx (U/mg protein) GR (U/mg protein), GST (µM/min/mg protein) specific enzyme activities in mice liver.

		GPx		
	Control	**Fucoidan**	**Sulfoxaflor**	**Sulfoxaflor+Fucoidan**
24h	4.09 ± 0.39^{bx}	5.01 ± 0.48^{ax}	4.39 ± 0.29^{bx}	4.26 ± 0.29^{bx}
7d	4.63 ± 0.29^{bx}	4.72 ± 0.87^{by}	5.46 ± 0.57^{ay}	$4.35 \pm 0.43^{by*}$
		GR		
	Control	**Fucoidan**	**Sulfoxaflor**	**Sulfoxaflor+Fucoidan**
24h	0.11 ± 0.01^{ax}	0.10 ± 0.01^{ax}	0.09 ± 0.02^{ax}	0.09 ± 0.01^{ax}
77d	0.10 ± 0.09^{ax}	0.11 ± 0.01^{bx}	0.10 ± 0.02^{bx}	0.11 ± 0.01^{bx}
		GST		
	Control	**Fucoidan**	**Sulfoxaflor**	**Sulfoxaflor+Fucoidan**
24h	302.48 ± 21.15^{ax}	261.08 ± 11.70^{by}	247.63 ± 10.02^{bcx}	213.04 ± 8.95^{cx}
7d	287.42 ± 19.20^{ax}	231.49 ± 7.06^{bx}	251.07 ± 19.66^{abx}	219.84 ± 14.59^{bx}

Values are expressed as mean ±standard error. Mean values bearing different superscripts in a row differ significantly (P≤0.05)

The differences between groups at the same duration are represented by the letters a, b, and c, and the differences between groups at different durations are represented by the letters x and y. At the P≤0.05 level, the data with different letters are statistically different (n = 8). Asterisks (*) show the differences between sulfoxaflor groups and sulfoxaflor+fucoidan groups.

Effects of Sulfoxaflor and Fucoidan on Oxidative DNA Damage (8-OHdG): The effects of sulfoxaflor and fucoidan on 8-OHdG levels are given in Figure 1. There was no statistically significant change in 8-OHdG levels at the end of 24 h in the liver treated with sulfoxaflor and fucoidan compared to the control. At the end of the 7-day treatment, sulfoxaflor was observed to significantly elevate 8-OHdG levels relative to the control group, whereas co-administration with fucoidan reduced 8-OHdG levels to those comparable with the control ($p \leq 0.05$).

Effects of Sulfoxaflor and Fucoidan on the Lipid Peroxidation (TBARS): Figure 2 shows the effect of sulfoxaflor and fucoidan on TBARS levels. At the end of 24 h, there were no significant changes in TBARS levels in the liver. TBARS levels in the sulfoxaflor-treated group were significantly higher than the control group, while TBARS levels in the sulfoxaflor+fucoidan-treated group were were decreased at the end of 7d. ($p \leq 0.05$).

Effects of Sulfoxaflor and Fucoidan on mRNA Expression of Target Genes: The effects of sulfoxaflor and fucoidan on XRCC1 mRNA expression levels are presented in Figures 3 and 4. Sulfoxaflor treatment significantly increased XRCC1 mRNA expression in the liver at both 24 hours and 7 days compared to the control group ($p \leq 0.05$). However, in the group treated with both sulfoxaflor and fucoidan, the increase in XRCC1 mRNA expression was significantly reduced compared to the sulfoxaflor-only group at both time points ($p \leq 0.05$).

Figures 5 and 6 demonstrated the effects of sulfoxaflor and fucoidan on APE1 mRNA expression. Sulfoxaflor significantly increased APE1 mRNA expression at the end of 24 h and 7 d compared to the control. Fucoidan significantly decreased APE1 mRNA expression levels in the sulfoxaflor-treated group at the end of 7 days ($p \leq 0.05$). The treatment groups did not exhibit any statistically significant differences in OGG1 and PARP1 gene expression levels in the liver.

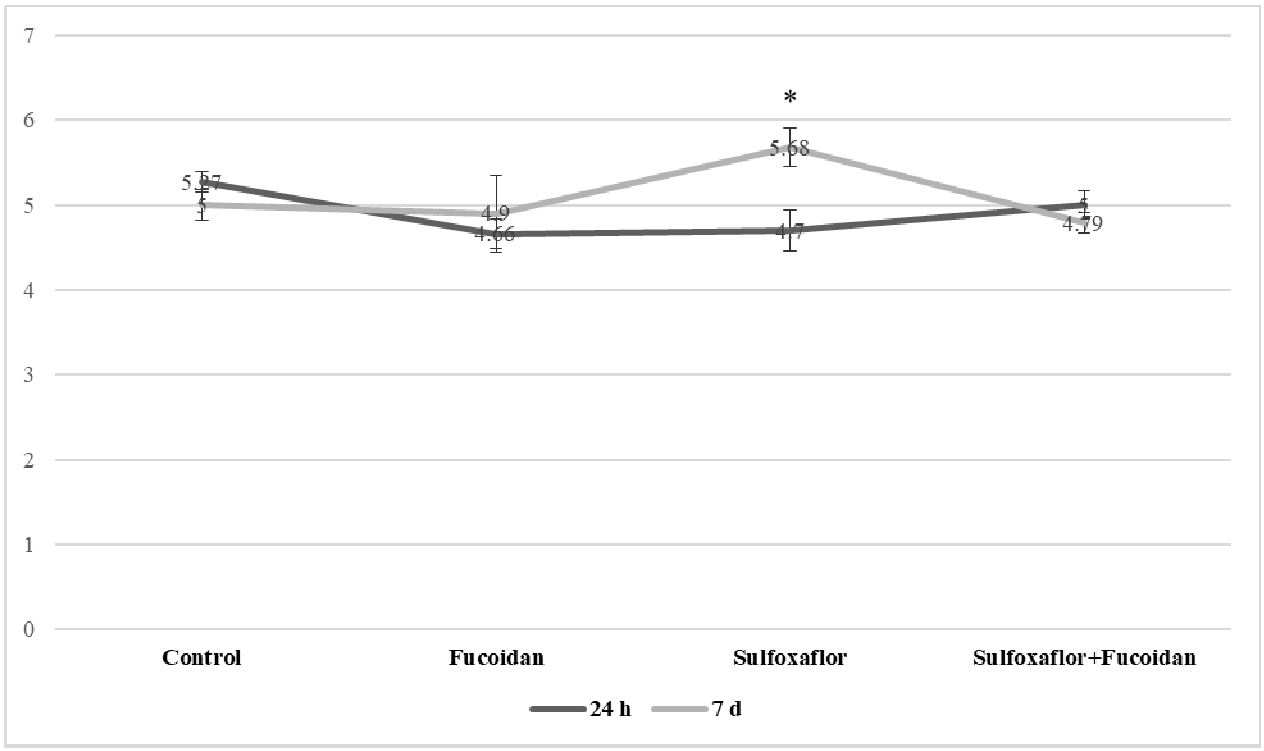

Figure 1. Changes in 8-OHdG levels in mice liver.
Values in figure are expressed as mean value and standart error.
Asterisks (*) show the differences between treatment groups and control.

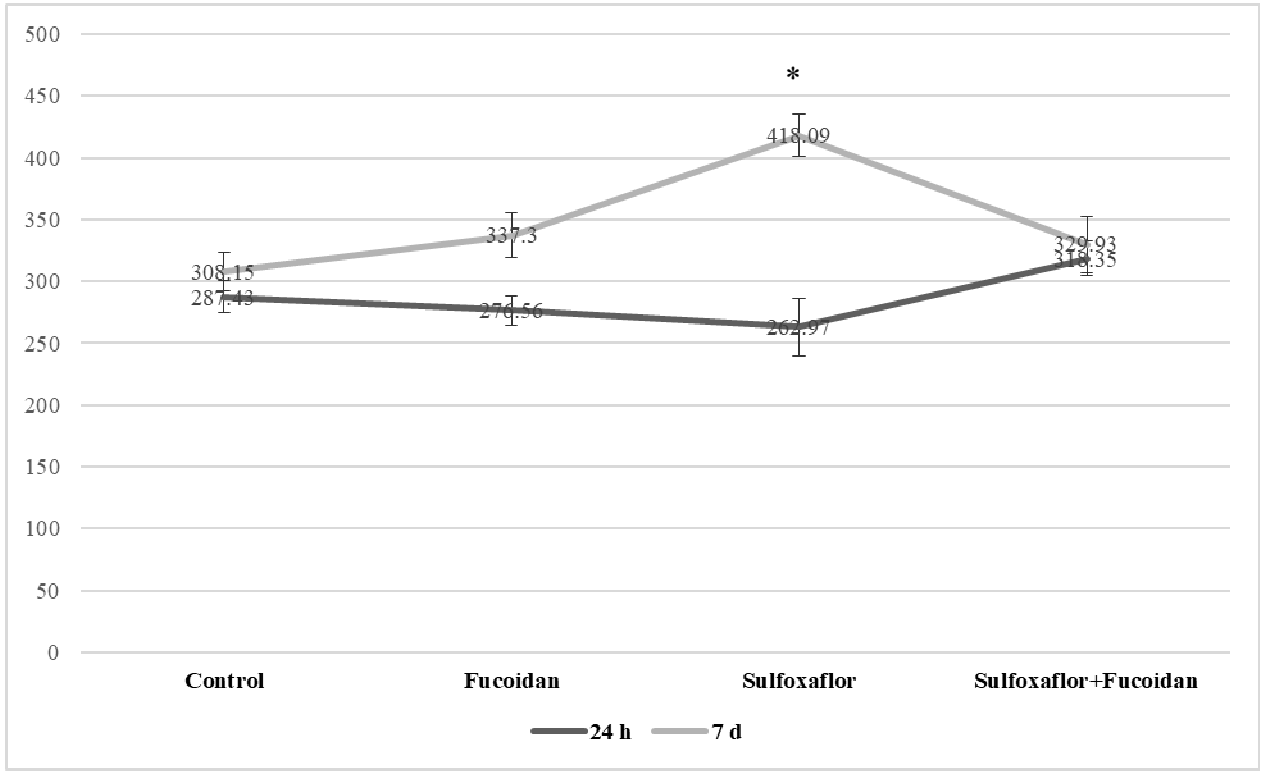

Figure 2. Changes in TBARS levels in mice liver.
Values in figure are expressed as mean value and standart error. Asterisks (*) show the differences between treatment groups and control.

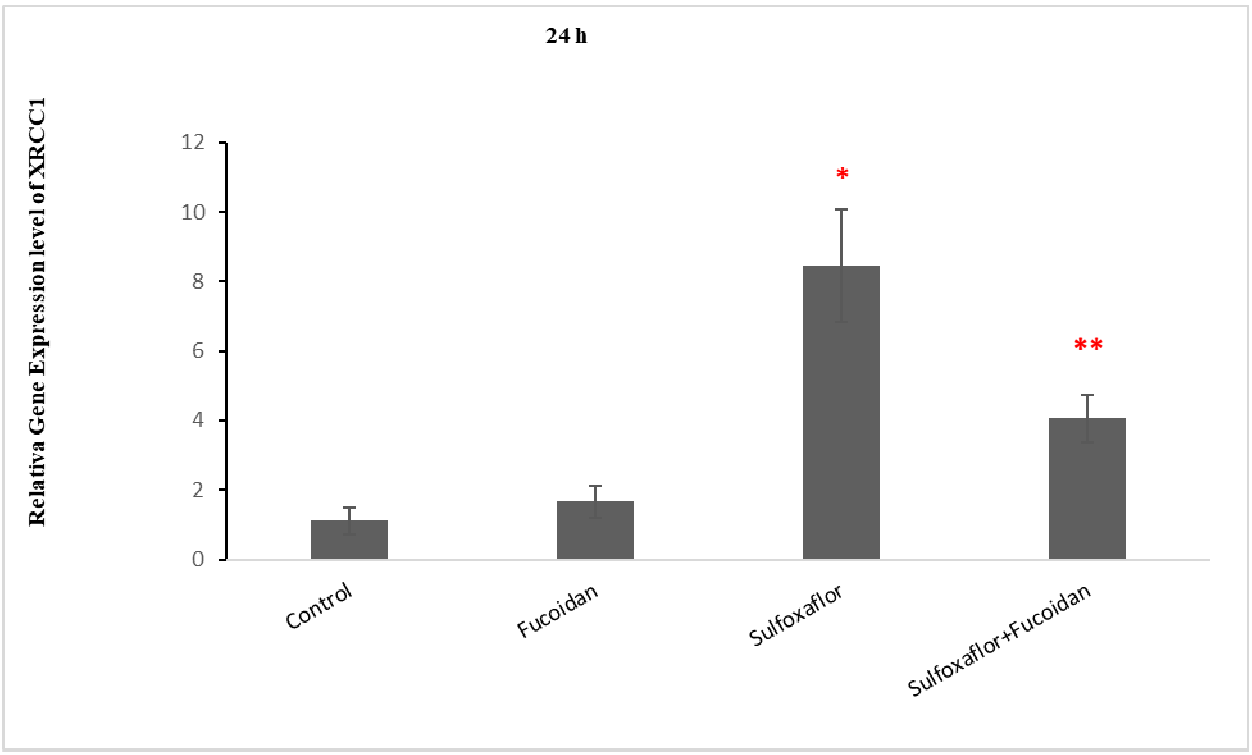

Figure 3. Fold changes (relative expression) in XRCC1 gene expression in mice liver at 24 h.
Values in figure are expressed as mean value and standart error of the mean.
Asterisks (*) show the differences between treatment groups and control group.
Asterisks (**) show the differences between sulfoxaflor group and sulfoxaflor+fucoidan group.

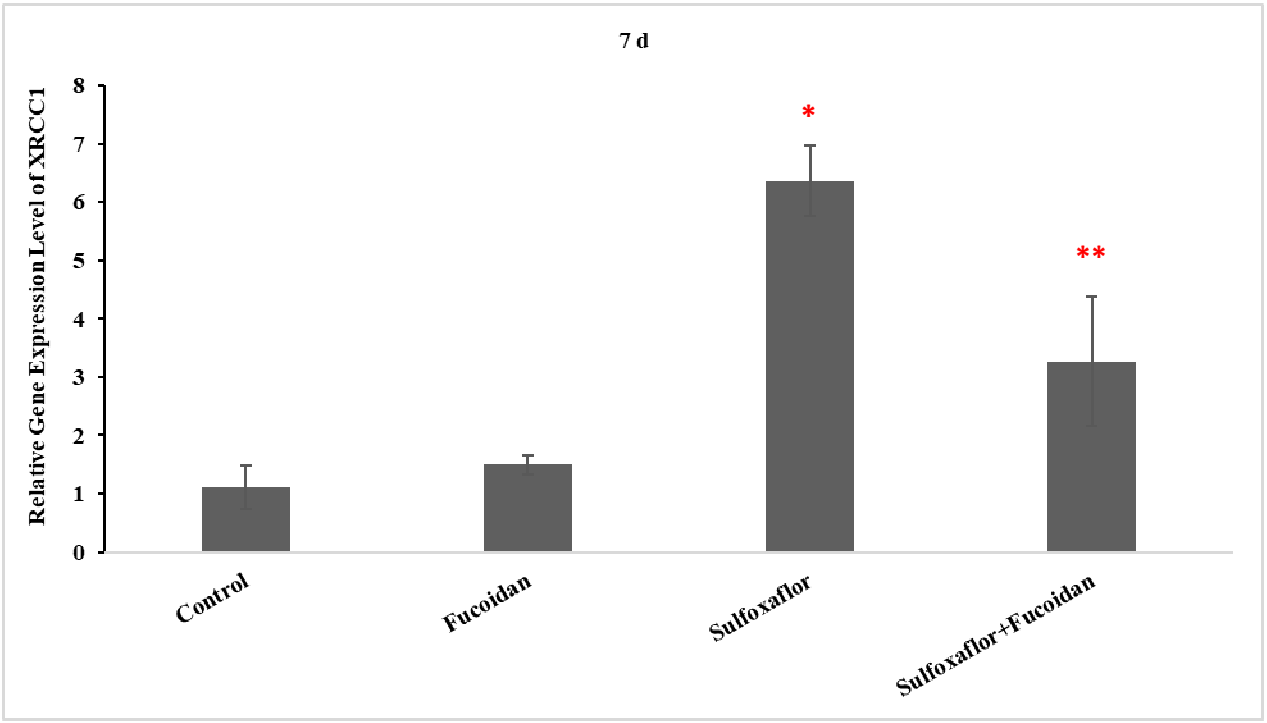

Figure 4. Fold changes (relative expression) in XRCC1 gene expression in mice liver at 7 d.
Values in figure are expressed as mean value and standart error of the mean.
Asterisks (*) show the differences between treatment groups and control group.
Asterisks (**) show the differences between sulfoxaflor group and sulfoxaflor+fucoidan group.

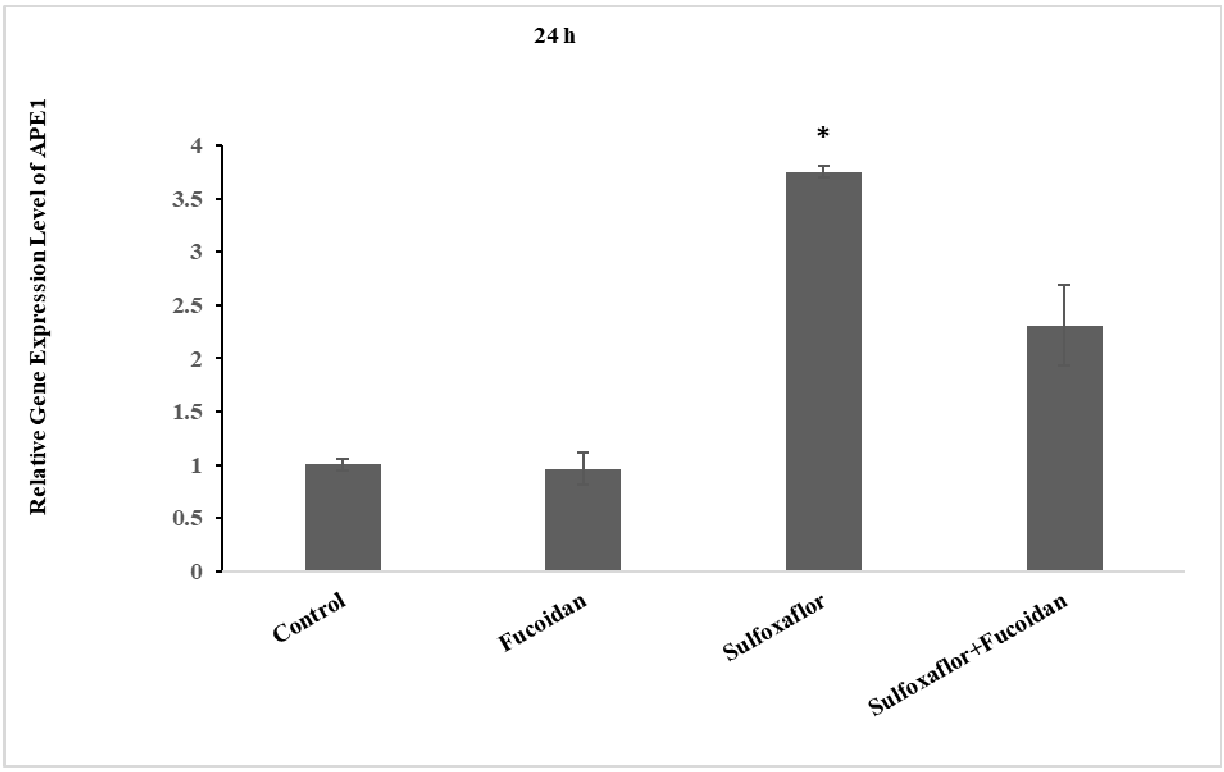

Figure 5. Fold changes (relative expression) in APE1 gene expression in mice liver at 24 h.
Values in figure are expressed as mean value and standart error of the mean.
Asterisks (*) show the differences between treatment groups and control group.

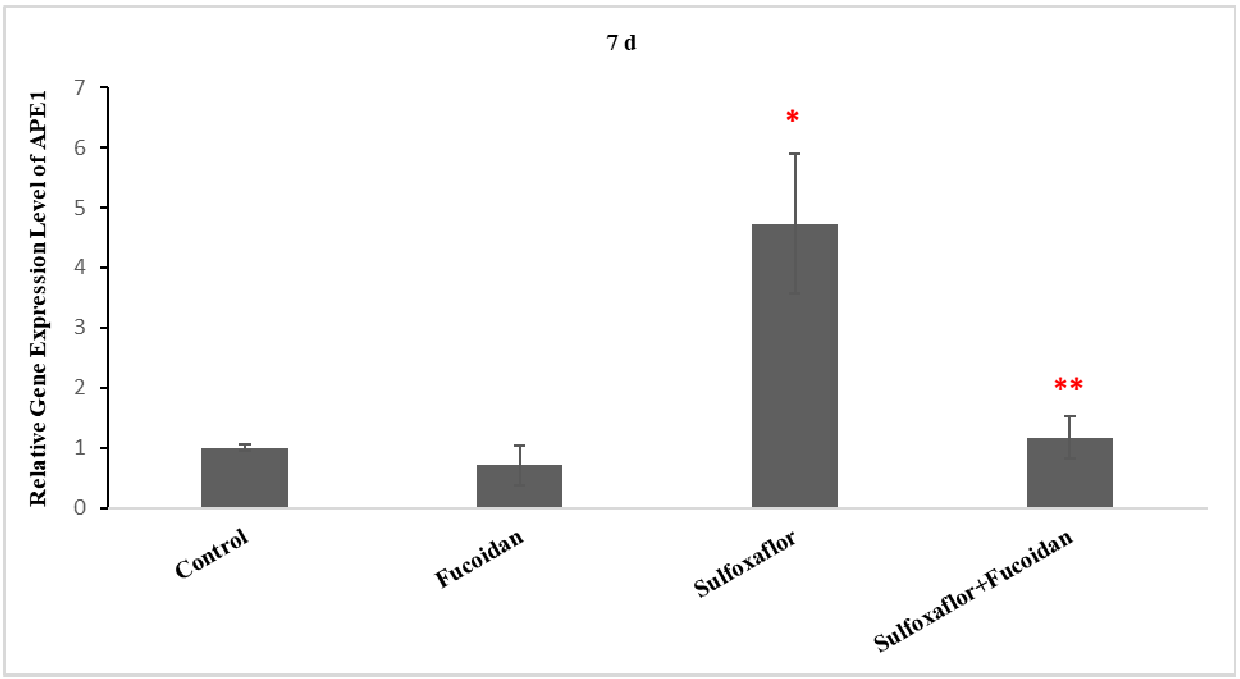

Figure 6. Fold changes (relative expression) in APE1 gene expression in mice liver at 7 d.
Values in figure are expressed as mean value and standart error of the mean.
Asterisks (*) show the differences between treatment groups and control group.
Asterisks (**) show the differences between sulfoxaflor group and sulfoxaflor+fucoidan group.

DISCUSSION

Neonicotinoids are a dominant class of broad-spectrum insecticides in the global market due to their unique drug targets and physicochemical characteristics (Bass *et al*., 2015). They are currently being studied as substitutes for various conventional insecticide classes (Simon-Delso *et al*., 2015). Neonicotinoids are widely distributed in the environment and accumulate across trophic levels (Alsafran *et al*., 2022). Numerous *in vitro* and *in vivo* studies have documented neonicotinoid toxicity, including hepatotoxicity/hepatocarcinogenicity, immunotoxicity, reproductive toxicity, neurotoxicity, multi-organ toxicity, and genetic toxicity (Han *et al*., 2018; Wang *et al*., 2018). As previously noted, the toxicity of neonicotinoids may be largely attributed to oxidative stress effects. Neonicotinoid insecticides are extensively studied for their role in oxidative stress through ROS production, which may lead to lipid peroxidation, membrane function alterations, DNA damage, and potentially mutagenic and carcinogenic effects (Wang *et al*., 2018). Recent studies indicate that fucoidan possesses antioxidant, anti-inflammatory, and immunomodulatory effects, offering protection against various toxic substances. This study demonstrates that sulfoxaflor induced oxidative stress by triggering lipid and oxidative DNA damage in the liver of male mice and altering the antioxidant system. It also upregulated the expression of DNA damage response genes (XRCC1, APE1) at the transcriptional level. Fucoidan provided considerable protection against sulfoxaflor-induced oxidative damage and regulated the transcriptional expression of DNA damage response genes (XRCC1, APE1).

The current findings showed that acute, sublethal sulfoxaflor exposure induces lipid and DNA damage by increasing TBARS and 8-OHdG levels and activating GSH-related antioxidants in the mice liver. Previously, we found that sulfoxaflor significantly elevated MDA, POC, 8-OHdG, and GSH levels in the serum of male mice and influenced TBARS, GSH-related antioxidants, and caspase-3 transcription levels in the brain (Piner Benli *et al*., 2021b; Piner Benli *et al*., 2021c). Studies on sulfoxaflor's reproductive and oxidative toxicity in rats have shown significant increases in FSH, LH, MDA, and GPx levels, along with elevated counts of dead and abnormal sperm and DNA damage in rat sperm (Mohamed *et al*., 2022). Another study reported that sulfoxaflor increased testicular MDA, NOx, and GSSG levels while decreasing GSH and cellular energy parameters in a dose-dependent manner. A notable increase in caspase-3 activity and DNA fragmentation was also observed (Said *et al*., 2021). Fathy *et al*. (2021) demonstrated that sulfoxaflor raised lipid peroxidation and SOD, CAT, and GPx enzyme activity in the thymus and spleen of rats.

GSH is a vital antioxidant, supporting detoxification by conjugating with molecules and acting as a critical cofactor for antioxidant enzymes such as GPx, GR, and GST. Changes in the GSH-related antioxidant system facilitate ROS removal and inhibit the oxidation of essential biomolecules like DNA, protein, and lipids (Hayes *et al*., 2005). Although many studies show that sulfoxaflor impacts antioxidant enzymes like SOD, CAT, and GPx, few studies address the relationship between GSH-dependent antioxidant systems and sulfoxaflor-induced oxidative stress (Piner Benli and Celik, 2021a; Piner Benli *et al*., 2021c). GST, an essential detoxifying enzyme, transforms various electrophilic chemicals into hydrophilic forms. As a second-stage detoxification enzyme, it catalyzes the conjugation of glutathione (GSH) with foreign compounds and their metabolites (Lushchak 2012). However, there is no evidence for GSH conjugation of sulfoxaflor in the detoxification process. The results show that GST-specific enzyme activity decreased in sulfoxaflor-treated groups compared to the control. Several studies have demonstrated that sulfoxaflor can interfere with GST catalytic activity, decreasing detoxification capacity and antioxidant function (Benli and Celik, 2021a; Zhou *et al*., 2023). This inhibition may have significant implications for xenobiotic detoxification, leading to increased oxidative stress, cellular damage, and potential toxicity.

Numerous xenobiotics can lead to genotoxic effects via direct DNA damage, indirect DNA damage, and epigenetic changes (Han *et al*., 2018; Ambreen *et al*., 2023; Al-Saeed *et al*., 2023; Ali *et al*., 2024). Several studies have assessed neonicotinoid genotoxicity by examining chromosomal aberrations (CAs), sister chromatid exchanges (SCEs), comet assays, and micronucleus tests (MN) *in vitro* and *in vivo* (Han *et al*., 2018). Neonicotinoid pesticides appear to be genotoxic in humans, rats, rabbits, and bovids. In *in vivo* studies, rabbits administered two doses of imidacloprid three times a week for four months showed genotoxic effects in both exposed groups based on MN frequency analysis (Stivaktakis *et al*., 2016). Thiacloprid, administered to rats in two doses over 30 days, caused considerable increases in micronuclei in BN cells. Female mice treated with imidacloprid at three doses over 24 hours showed significantly reduced mitosis and increased micronucleus production (Kataria *et al*., 2016). In nuclear and mitochondrial DNA, 8-OHdG is a predominant form of accessible radical-induced oxidative lesions and is widely used as an oxidative stress biomarker (Valavanidis *et al*., 2009). This lesion's repair is initiated by 8-oxo guanine glycosylase (OGG1) in the nucleus and mitochondria. XRCC1, APE1, PARP1, DNA ligase III, DNA polymerase γ, and Exonuclease G (EXOG) are additional

enzymes involved in the DNA repair mechanism (Van Houten *et al*., 2018). Although the current study observed an increase in 8-OHdG levels, sulfoxaflor did not affect OGG1 and PARP1 mRNA expression. However, sulfoxaflor upregulated XRCC1 and APE1 mRNA expression in the livers of mice. This is the first study to show that sulfoxaflor induces oxidative DNA damage and activates DNA damage genes at the transcriptional level. Sulfoxaflor has been shown to induce genotoxicity and apoptosis in human blood lymphocytes via oxidative stress-associated DNA damage (Sınacı *et al*., 2023). Previous studies have demonstrated neonicotinoids' genotoxic effects by inducing DNA damage. The neonicotinoid insecticide clothianidin exhibited cytotoxic and genotoxic effects at high concentrations in human peripheral blood lymphocytes (Atli Sekeroglu *et al*., 2019). Ge *et al*. (2015) found that imidacloprid stimulated DNA damage via oxidative stress in the zebrafish liver.

Fucoidan, a biologically essential compound from macroalgae species, has been widely studied for its potential bioactivity (Fitton *et al*., 2015; Abdel-Latif *et al*., 2022). Studies have demonstrated fucoidan's protective effects against specific pathologies, including liver disease (Li *et al*., 2020), brain injury (Wang *et al*., 2021), viral infections (Pradhan *et al*., 2022), and inflammatory disease (Sanjeewa *et al*., 2021), showing marked antioxidant activity. With growing interest in natural antioxidants, fucoidans have emerged as highly valued nutrients and effective, safe therapeutics against various diseases (Li *et al*., 2020). Fucoidan's role in oxidative stress, lipid and carbohydrate metabolism, inflammatory response, tumor growth, and metastasis has become a focus of recent studies. Fucoidan's liver-protective effects include antioxidant activity (Dimitrova-Shumkovska *et al*., 2020), anti-inflammatory effects (Wang *et al*., 2024), fibrosis regulation (Hayashi *et al*., 2008), antiviral activity against hepatitis B virus (Li *et al*., 2017), and liver regeneration enhancement (Wang *et al*., 2018). The antioxidant capacity of fucoidan has been widely studied *in vivo* (Jhamandas *et al*., 2005; Heeba and Morsy, 2015; Meenakshi *et al*., 2016; Abdel-Daim *et al*., 2020). Fucoidan protects against oxidative stress by enhancing antioxidant enzyme activity and reducing ROS generation (Wang *et al*., 2018; Kim *et al*., 2019a; Kim *et al*., 2019b). It also boosts antioxidative capacity by upregulating heme oxygenase-1 and superoxide SOD-1 and increasing levels of glutathione (GSH), SOD, and catalase (CAT) (Ryu *et al*., 2016; El-Boshy *et al*., 2017). Consistent with these reports, the current study shows that fucoidan protected liver cells from oxidative lipid and DNA damage by enhancing GSH and GSH-related antioxidant enzyme activities through its potent antioxidant capacity. Additionally, fucoidan regulated DNA damage response genes by influencing APE1 and XRCC1 mRNA expression in the sulfoxaflor-exposed mice liver. These findings suggest that fucoidan may exert its protective effect by re-regulating DNA damage response genes at the transcriptional level.

Conclusion: Our findings show that sulfoxaflor has oxidative potential by increasing DNA oxidation and lipid peroxidation in mice liver. The antioxidant system may reduce oxidative effects via scavenging sulfoxaflor-generated ROS. The upregulation of DNA damage repair genes may be related to sulfoxaflor-induced DNA damage. Fucoidan protected liver cells from sulfoxaflor-induced oxidative effects, and it regulated DNA damage response at the transcriptional level in mice liver. The present study is the first report elucidate the molecular mechanism of sulfoxaflor-induced damage and the regulatory role of fucoidan on oxidative effects and DNA damage response in the liver. Further studies are needed to elucidate the cytotoxic, genotoxic, and carcinogenic effects of sulfoxaflor and the protective role of fucoidan on these aspects.

Ethics Approval The Ethics Committee of the Cukurova University Faculty of Medicine Experimental Medicine Research and Application Center (Approval Code: 9, Approval Date: 04.11.2019) approved this study.

Consent to Participate Not applicable

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

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

Funding This research was supported financially by the Cukurova University Scientific Research Commission (TSA-2021-13246).

Authors' Contributions P.P.B; conceived and designed the analyses, collected data, performed the analyses, drafted manuscript, critically revised manuscript, Y.K.D; conceived and designed the analyses, critically revised manuscript, C.C; conceived and designed the analyses, collected data, performed the analyses, critically revised manuscript.

Acknowledgements The authors thank the Biotechnology Research and Application Center, Faculty of Medicine, Faculty of Engineering, and Department of Food Engineering at Cukurova University.

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