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Original article

Ameliorative potential of mustard greens (*Brassica juncea*) leaf extract on aflatoxin B1-induced toxicity: An in vivo study in rats

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Abstract

The present study was conducted to evaluate the ameliorative effect of *Brassica juncea* (BJ) leaves methanol extract against aflatoxin B_1 (AFB_1) induced toxicity in rats. Thirty-six male albino rats, six weeks old (weighing 140-190 g), were randomly assigned into 6 groups (n=6). AFB_1 (200 µg/kg b.w., orally) was given to rats on alternate days and *Brassica juncea* extract (BJE) (300 and 600 mg/kg b.w., orally) in combination with AFB_1 on successive days for 28 days. AFB_1 exposure significantly elevated hepatic and kidney function parameters. Moreover, AFB_1 markedly reduced antioxidant enzymes, elevated malondialdehyde (MDA) levels and altered the gene expression of the NF-E2-related factor 2 (Nrf2) gene and the caspase-3 gene, promoting redox stress and apoptosis. Co-administration of silymarin (100 mg/kg b.w.) and BJE (300 and 600 mg/kg b.w.) significantly restored the liver. lung and kidney tissue biochemical markers, enhanced antioxidant enzyme activities and reduced pro-inflammatory cytokines (IL-6, TNF-α). Additionally, BJE significantly ameliorated the mitochondrial dysfunction, redox imbalance and tissue damage caused by AFB_1. BJE significantly upregulated the mRNA and protein expression levels of the Nrf2 gene while downregulating the expression of cleaved caspase-3. Therefore, the results clearly indicate that BJ could potentially alleviate AFB_1-induced toxicity due to its antioxidant, anti-apoptotic and anti-inflammatory properties.

Keywords: aflatoxin B1, *Brassica juncea*, caspase-3, kidney, liver, lung, Nrf2, oxidative stress, toxicity

Introduction

Aflatoxins are secondary metabolites belonging to the class of mycotoxins produced by *Aspergillus flavus* and *Aspergillus parasiticus* species. These toxins frequently contaminate food and feed products, which has led to an increased rate of death and illness in both humans and animals (Rushing and Selim 2019). Moreover, continual exposure to aflatoxin B_1 (AFB_1) is linked to lung adenocarcinoma, aflatoxicosis and hepatocellular carcinoma, making it a public health concern worldwide (Ajmal et al. 2022). AFB_1, AFB_2, AFM_1, AFM_2, AFG_1 and AFG_2 are most commonly detected in food and feed commodities. Among these mycotoxins, AFB_1 is recognized as a Group 1 carcinogen by the International Agency for Research on Cancer (IARC). Prolonged exposure can result in delayed development, impaired metabolic system and immunosuppression (Mohammad et al. 2017). Following metabolism in the liver by cytochrome P450 enzymes, AFB_1 is converted into reactive oxygen species (ROS), which can form DNA and protein adducts, causing tissue damage. The key mechanism underlying toxicity involves the induction of oxidative injury (Moloi et al. 2024). AFB_1 downregulates the expression of Nuclear factor erythroid 2-related factor 2 (Nrf2), while activating Bcl-2-associated X protein (BAX), which alters mitochondrial membrane permeability. The increased permeability leads to the release of cytochrome c, forming an apoptosome complex. This activates the executioner caspase-3, resulting in apoptosis (Xu et al. 2021). AFB_1 can cause tissue and organ damage by increasing malondialdehyde (MDA) levels and depleting the antioxidant defense system [glutathione (GSH), catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx)] (Li et al. 2022). Several studies have evaluated the ameliorative potential of phytochemicals against aflatoxin-induced toxicity, including curcumin, flavonoids, alkaloids, phenols and lycopene (Sharma and Patial 2021). *Brassica juncea* (BJ), commonly known as green, brown and vegetable mustard, belongs to the Brassicaceae family (Shankar et al. 2019). It originates from Central Asia and is also cultivated in China, India, Nepal, Pakistan and Japan. BJ contains several bioactive compounds such as sterols, flavonoids, alcohols, glycosides and phenolic compounds. Several studies have demonstrated that BJ has anticancer, antioxidant, antidiabetic, antimicrobial and anti-inflammatory effects (Kumar et al. 2011). However, the protective potential of BJE has not been studied against AFB_1-induced toxicity in the liver, kidney and lungs. Hence, the present study aimed to evaluate the protective potential of BJE (BJ extract) against AFB_1-induced toxicity in a rat model.

Materials and Methods

Procurement of chemicals

All the solvents and chemicals used throughout the experiment were of analytical purity. Methanol and AFB_1 were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). Silymarin (Abbott Laboratories Pak Ltd) was procured from a local pharmacy in Lahore.

Extract preparation

BJ leaves were thoroughly washed and air-dried in the shade at 26°C for 7 days. The dried powder leaf sample was suspended in 98% methanol (1:10 w/v), placed in a shaking incubator at room temperature and agitated at 120 rpm for 72 hrs. The extract was filtered using Whatman No. 1 filter paper. Subsequently, the filtrate was concentrated at 50°C using a rotary vacuum evaporator and stored at 4°C until further use. Before the experimental trial, the methanolic extract was evaluated for its nutritional composition (AOAC 2000), phytochemical profiling (Harborne 1998) and antioxidant activity (Grzegorczyk et al. 2007).

Experimental design

Thirty-six male albino rats (140-190 g, aged 6 weeks) were randomly divided into 6 groups and kept at 24±2°C in a 12-hour light-dark cycle with unrestricted access to food and water. For inducing toxicity, AFB_1 (200 µg/kg b.w.) was dissolved in corn oil (1:1 v/v) and given via oral gavage on alternate days for 28 days. During the experimental trial, the animals were weighed weekly. The BJE treatments, suspended in normal saline and silymarin, were administered daily by oral gavage. The dosage of BJE was selected based on a review of previous in vivo studies (Kim et al. 2003, Valavala et al. 2011, Saleem et al. 2021) and further adjusted in a preliminary pilot study to ensure both safety and efficacy. The rat groups were as follows: Group I: normal control group received normal saline; Group II: vehicle control group received 1ml corn oil; Group III: AFB_1 treated group received AFB_1 (200 µg/kg b.w.); Group IV: Standard group: AFB_1 + silymarin (100 mg/kg b.w.); Group V: Treatment group 1 (AFB_1 + BJE 300 mg/kg b.w.); Group VI: Treatment group 2 (AFB_1 + BJE 600 mg/kg b.w.). The experimental procedure was approved by the Institutional Guidance of the Ethical Review Committee of the University of Veterinary and Animal Sciences, Lahore (Reg no. DR/ 533; December 2023).

Table 1. Primer sequence of Nrf-2 and Caspase-3.

Gene	Forward Primer	Reverse Primer
Caspase-3	GGAGCTTGGAACGCGAAGA	ACACAAGCCCATTTCAGGGT
Nrf2	TTGTAGATGACCATGAGTCGC	TGTCCTGCTGTATGCTGCTT

Table 2. Weight interpretation of rats in a 28-day AFB_1-induced toxicity study.

Groups	Initial Weight (g)	Final Weight (g)	Relative liver weight (g)	Relative Kidney weight (g)	Relative Lung weight (g)
Control	$172.33±6.11^{abc}$	$203.67±7.64^{a}$	$2.68±0.23^{ab}$	$1.19±0.17^{a}$	$0.66±0.19^{a}$
Corn oil	$169.33±5.13^{abc}$	$199±6.56^{a}$	$2.66±0.21^{ab}$	$1.16±0.15^{a}$	$0.69±0.14^{a}$
AFB_1	$180.33±6.43^{ab}$	$198.67± 4.5^{a}$	$1.99±0.11^{c}$	$0.65±0.28^{b}$	$0.52±0.17^{a}$
Silymarin	$161.33±5.69^{c}$	$193.33±5.77^{a}$	$2.98±0.21^{a}$	$1.25±0.19^{a}$	$0.67±0.1^{a}$
BJE 300	$183.33±4.93^{a}$	$204±6.08^{a}$	$2.32±0.15^{bc}$	$0.83±0.14^{ab}$	$0.46±0.17^{a}$
BJE 600	$166.67±5.86^{bc}$	$197±5.2^{a}$	$2.6±0.25^{ab}$	$1.09± 0.10^{ab}$	$0.6±0.07^{a}$

Experimental data were recorded as mean ± S.D with (n=6) rats per group. Mean values denoted with distinct alphabets display significant difference ($p<0.05$).

Blood sampling, biochemical and oxidative stress biomarker analysis

At the end of the experiment, followed by overnight fasting, the rats were euthanized under Isoflurane anesthesia. Blood samples were collected via cardiac puncture and serum was separated using a low-speed centrifuge at 3500 g, 4°C for 20 min. Biochemical biomarkers were measured using Abcam and Elabscience® kits, while enzyme activity levels were quantified with commercial kits using a Response® 910 blood chemistry analyzer (Diagnostic System-SIEMENS, Munich, Germany). Tissue samples were homogenized in phosphate buffer saline (PBS, pH 7) and centrifuged at 3000 rpm for 10 min at 4°C. The resulting clear supernatant was kept at -80°C until analysis. Oxidative injury biomarkers were measured using Elabscience® Biochemical Assay kits as per instructions.

Quantitative Real-Time PCR (qRT-PCR) and western blotting

The gene expression of Nrf2 and Caspase-3 was determined using a StepOne Plus thermal cycler (Applied Biosystems, USA), according to the protocol described by Zhao et al. (2019). Total RNA was extracted from each tissue sample using Trizol reagent (No. 9109 Takara, Japan) and quantified using a GeneQuant 1300 GE spectrometer (USA). Samples having an absorbance ratio between 1.8 and 2.0 at 260/280 nm were deemed acceptable for reverse transcription. cDNA analysis was performed using a RevertAid First Strand cDNA Synthesis Kit (Thermo Scientific). The $2^{-\Delta\Delta CT}$ formula was used to quantify gene expression using β-actin as a reference gene. Premier 5.0 software was used for primer design (Table 1). For western blotting, the protein expression of Nrf2 and Caspase-3 genes was evaluated, following the method described by Wang et al. (2020). Enhanced chemiluminescence (ECL) was used for band visualization and protein expression was quantified using ImageJ 1.43 software, with β-actin serving as a loading control for normalization.

Data analysis

SPSS version 26 (Statistical Package for Social Sciences; SPSS Inc., Chicago, IL, USA) was used to analyze the data. Data were represented as mean ± standard deviation (SD). One-way ANOVA with Tukey's post hoc test was used with a statistical significance of $p≤0.05$.

Results

Protective effect of methanolic extract of BJE on organ and body weight

The rats were weighed weekly throughout the 28-day trial. Table 2 shows that while all groups displayed weight gain over time, the AFB_1-treated group displayed a significant decrease in final body weight (198.67±4.5 g) compared to the control (203.67±7.64 g) and treatment groups. AFB1 administration also significantly reduced the relative liver and kidney weights ($p<0.05$). Treatment groups (BJE at 300 and 600 mg/kg b.w.) improved the respective organ weights.

Table 3. Serum levels of inflammatory mediators in the study rat groups.

Serum level	Control	Vehicle control	AFB_1	Silymarin	AFB_1+BJE 300	AFB_1+BJE 600
TNF-α (ng/l)	25.49 ± 4.7^{c}	24.26 ± 3.65^{c}	125.8 ± 5.38^{a}	48.53 ± 2.78^{b}	56.81 ± 9.31^{b}	50.4 ± 5.63^{b}
IL-6 (ng/l)	53.71 ± 6.49^{c}	53±6.4c	131.92 ± 6.61^{a}	81.43 ± 6.85^{b}	91.58 ± 5.07^{b}	83.42 ± 5.37^{b}

Experimental data were recorded as mean ± S.D. with six rats per group. Mean values denoted with distinct letters in each column indicate statistically significant difference ($p<0.05$).

Table 4. Impact of *Brassica juncea* extract (BJE) on hepatic and kidney biomarkers in the AFB_1-induced hepatotoxicity and nephrotoxicity.

Groups	AST (U/L)	ALT (U/L)	ALP (U/L)	TP (mg/dL)	LDH (IU/L)	TG (mg/dL)	Cho (mg/dL)	Creatinine (mg/dL)	Urea (mg/dL)	Uric Acid (mg/dL)
Control	152.25 ± 4.12^{c}	66.36 ± 1.43^{cd}	163.33 ± 5.24^{cd}	6.13 ± 0.09^{b}	1028.24 ± 7.45^{b}	34.99 ± 2.18^{cd}	70.87 ± 3.83^{c}	0.51 ± 0.01^{d}	49.02 ± 1.73^{b}	1.31 ± 0.14^{b}
Corn oil	150.33 ± 4.04^{c}	64.31 ± 1.4^{d}	162.7 ± 4.21^{d}	6.09 ± 0.16^{b}	1028.27 ± 7.03^{b}	34.19 ± 1.62^{d}	70.96 ± 2.61^{c}	0.5 ± 0.01^{d}	49.14 ± 1.38^{b}	1.23 ± 0.11^{b}
AFB_1	215.07 ± 6.18^{a}	93.99 ± 1.93^{a}	205.65 ± 6.72^{a}	4.13 ± 0.2^{c}	1321.74 ± 9.75^{a}	92.28 ± 3.32^{a}	155.94 ± 2.38^{a}	0.63 ± 0.01^{a}	67.82 ± 1.27^{a}	1.86 ± 0.16^{a}
Silymarin	161.17 ± 4.91^{bc}	70.25 ± 1.03^{bc}	175.71 ± 3.6^{bc}	6.52 ± 0.11^{ab}	1043.58 ± 8.46^{b}	38.94 ± 1.27^{cd}	82.69 ± 2.3^{b}	0.54 ± 0.01^{c}	43.82 ± 1.86^{c}	1.39 ± 0.17^{b}
BJE 300	170.62 ± 4.54^{b}	74.67 ± 3.01^{b}	188.06 ± 3.05^{b}	6.89 ± 0.29^{a}	1048.2 ± 9.49^{b}	50.5 ± 1.27^{b}	89.25 ± 1.52^{b}	0.57 ± 0.01^{b}	50.97 ± 1.48^{b}	1.49 ± 0.14^{ab}
BJE 600	166.66 ± 4.53^{b}	72.62 ± 1.22^{b}	178.29 ± 3.21^{b}	6.68 ± 0.4^{ab}	1041.04 ± 8.51^{b}	39.99 ± 0.63^{c}	82.88 ± 3.3^{b}	0.55 ± 0.01^{bc}	44.28 ± 1.77^{c}	1.46 ± 0.15^{ab}

Data are presented as mean ± SD with six rats per group. Mean values labeled with distinct letters within each column indicate significant difference ($p<0.05$).

Protective effect of methanolic extract of BJE on pro-inflammatory cytokines in AFB_1-induced hepatotoxicity

Oral gavage administration of AFB_1 on alternate days resulted in significant elevation of proinflammatory cytokines, including TNF-α and IL-6 (Table 3). However, co-administration of Silymarin and BJE extract (BJE 300 and BJE 600 mg/kg b.w.) significantly restored the levels of proinflammatory cytokines.

Protective effect of methanolic extract of BJE on hepatic and kidney biomarkers in AFB_1-induced toxicity

The protective potential of BJE on hepatic and kidney function biomarkers in AFB_1-induced toxicity was evaluated by assessing the levels of Total protein (TP), Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), Lactate dehydrogenase (LDH), Alkaline phosphatase (ALP), Triglycerides (TG), Cholesterol (Cho), creatinine, urea and uric acid (Table 4). AFB_1 exposure significantly increased the levels of AST, ALT, ALP, LDH, TG, Cho, creatinine, urea and uric acid, while TP levels were decreased compared to the control group ($p<0.05$). Co-administration with BJE at 300 and 600 mg/kg b.w. markedly modulated the elevated levels of hepatic and kidney biomarkers in a dose-dependent manner.

Protective effect of methanolic extract of BJE on oxidative stress and antioxidant status of AFB_1-induced toxicity in liver, kidney and lungs

To further evaluate the protective effect of BJE, oxidative damage biomarkers and antioxidant defense parameters including MDA, GSH, CAT, SOD and GPx were assessed (Fig. 1). AFB_1 exposure significantly elevated MDA levels: 40.32 nmol/g (liver), 25.92 nmol/g (kidney) and 4.72 nmol/g (lung), indicating enhanced lipid peroxidation and oxidative damage ($p<0.05$). AFB_1 markedly impaired the antioxidant defense system ($p<0.05$) in respective organs compared to the control group, indicating toxicity. BJE (300 and 600 mg/kg b.w.) reduced MDA levels and restored antioxidant enzymes, demonstrating a protective effect against redox imbalance.

Protective effect of methanolic extract of BJE on Nrf2 and Caspase-3 expression in AFB_1-induced toxicity in liver, kidney and lungs

AFB_1 exposure significantly downregulated Nrf2 expression, indicating compromised antioxidant defense and upregulated Caspase-3 expression, reflecting enhanced apoptosis. Treatment with silymarin and BJE (300 and 600 mg/kg b.w.) significantly increased Nrf2 expression and decreased Caspase-3 gene expression in a dose-dependent manner as shown in Fig. 2. The protein expression patterns correlated with qRT-PCR results.

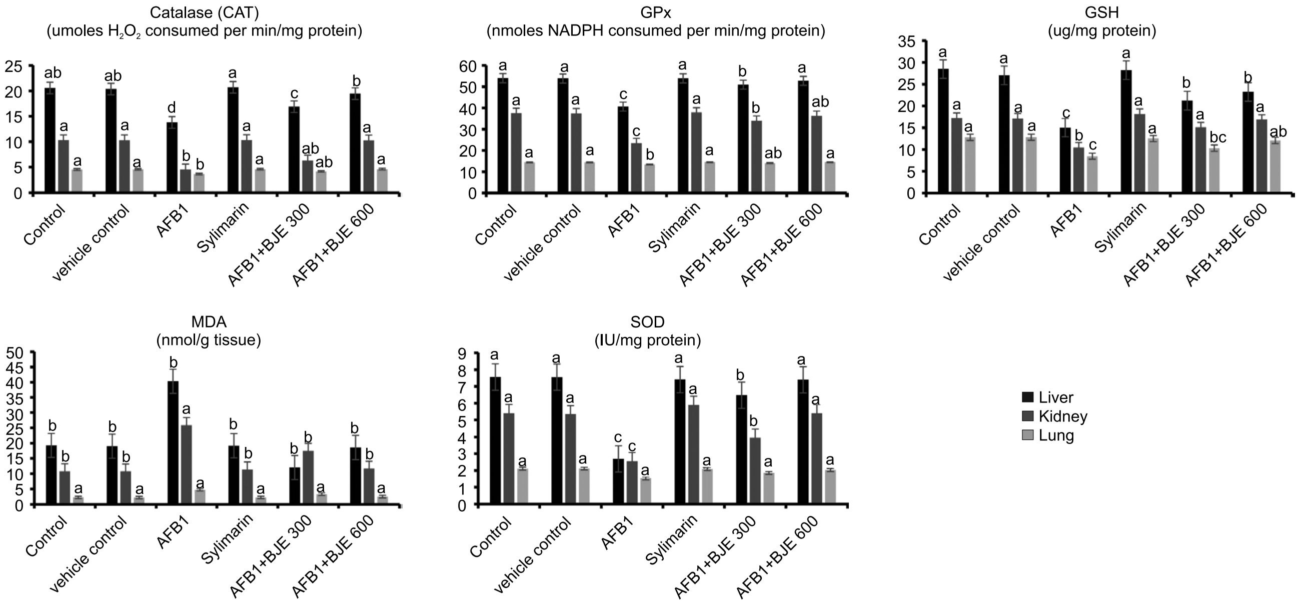

Fig. 1. Protective effect of BJE on oxidative injury and antioxidant system in liver, kidney and lung tissue. Experimental data were recorded as mean ± SD. Bars with distinct letters indicate significant difference ($p<0.05$).

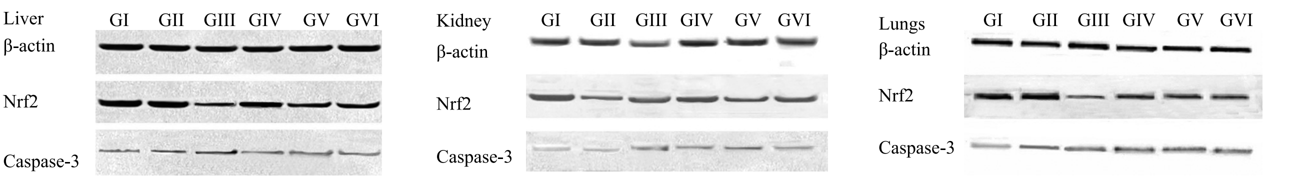

Fig. 2. Western blot analysis of β-actin, Nrf2 and Caspase-3 protein expression in liver, kidney and lung tissues across six rat groups: G1 (normal control), G2 (vehicle control), G3 (AFB_1-treated), G4 (AFB_1 + silymarin), G5 (AFB_1 + 300 mg/kg BJE) and G6 (AFB_1 + 600 mg/kg BJE). The expression of target proteins was normalized using β-actin as a loading control.

Discussion

Aflatoxins are naturally occurring mycotoxins produced by *Aspergillus flavus* and *Aspergillus parasiticus* fungal species. These toxins contaminate food and feed commodities and are associated with various types of cancers and aflatoxicosis both in human and animals. To date, more than 18 different types of aflatoxins have been identified (Benkerroum 2020). Humans and animals are continuously exposed to harmful dietary contaminants such as mycotoxins, which highlights the urgent need for an antidote that could potentially ameliorate the effects of AFB_1. For this purpose, numerous studies have explored the protective effects of various phytochemicals, including flavonoids, saponins, polyphenols and alkaloids, in counteracting the toxicity induced by AFB_1. BJ is an abundant source of bioactive compounds, particularly alkaloids, sterols, flavonoids and polyphenols. To our knowledge, no study has been conducted on the protective effect of BJE on AFB_1 induced toxicity, although other toxicological studies have been reported. Therefore, the present study aimed to evaluate the ameliorative effect of BJE at two distinct doses (300 and 600 mg/kg b.w.) in mitigating the AFB_1-induced toxicity in a rat model. The findings of the present study validated the protective potential of BJE against AFB_1-induced toxicity by restoration of hepatic enzyme levels, improving kidney function parameters, reducing lung tissue damage and modulation of antioxidant enzymes with expression of Nrf2 and caspase-3 gene in tissues. In the current study, AFB_1 exposure reduced feed intake in rats and led to a significant decline in body weight (Table 2), aligning with previous findings (Subramaniam et al. 2022). Studies have shown that AFB_1 exposure is associated with reduction in leptin levels, causing damage to the epithelial lining of the gastrointestinal tract, affecting body weight and energy balance (Wang et al. 2010). However, treatment with BJE (300 and 600 mg/kg b.w.) significantly relieved the AFB_1-induced alteration in the present study. Specifically, BJE 600 mg/kg b.w. displayed strong protective potential comparable to that of Silymarin in maintaining energy metabolism and preventing weight loss. Liver damage caused by AFB_1 was confirmed by significant elevation in serum AST, ALP, LDH, ALT, TG and cholesterol levels, indicating the successful establishment of the AFB_1 toxicity model in rats (Table 4). AST, ALT, TG, Cho and LDH enzymes are produced in liver cells and their release into the blood is an indication of liver damage. The rise in LDH, TG and Cho levels was indicative of hepatic parenchymal cell injury and a decrease in TP was suggestive of liver necrosis as well as kidney dysfunction. The results of liver damage were comparable with previous findings (Chen et al. 2019, Hua et al. 2021, Karaca et al. 2021, Wu et al. 2022, Lin et al. 2023, Liu et al. 2023, Duan et al. 2025). The observed elevation in kidney function biomarkers (urea, uric acid and creatinine) indicates impaired epithelial transport in collecting tubules, proximal tubule dysfunction and compromised filtration, which contributes to waste accumulation in the blood. These changes may also result from necrotic damage to the renal parenchyma (Gowda and Ledoux 2008). The observed kidney damage in this study (Table 4) was comparable to the results reported in prior investigations (Owumi et al. 2020, Al-Shahari et al. 2022, Wang et al. 2022, İrak et al. 2024, Ismail et al. 2024). On the other hand, treatment with BJE 600 mg/kg b.w. has been shown to improve liver and kidney function parameters. In the liver, cytochrome P450 monooxygenases convert AFB_1 into AFB_1-8,9-exo-epoxide (AFBO), generating ROS that may target mitochondria, inducing Lipid Peroxidation (LPO). MDA is a terminal byproduct of LPO and its elevated levels indicate oxidative damage (Cordiano et al. 2023). The rise in MDA levels in liver, kidney and lung tissue aligns with previously reported data (Cheng et al. 2023, Ismail et al. 2024). To counteract ROS and maintain cellular homeostasis, the body's intrinsic antioxidant defense mechanisms, including SOD, CAT and GPx, are activated. The SOD enzyme converts superoxide radicals to hydrogen peroxide. CAT catalyzes the conversion of hydrogen peroxide to water and oxygen. GPx maintains cellular redox homeostasis by scavenging ROS using GSH as a cofactor in reducing hydrogen and lipid peroxides (Pei et al. 2023). Liver, kidney and lung tissue from AFB_1-treated rats exhibited a significant decline in intrinsic antioxidant defense system including SOD, GPx, GSH and CAT due to overwhelming oxidative damage. The reduction in GSH levels was possibly due to the conjugation of NAPQ1 to GSH, leading to mercapturic acid formation. The trend in the antioxidant status of liver, kidney and lung tissue in the present study was consistent with those reported previously (Yilmaz et al. 2018, El-Mekkawy et al. 2020, Gugliandolo et al. 2020, Gao et al. 2021, Hua et al. 2021, Mostafa et al. 2021, Hassaneen et al. 2023, Albadrani et al. 2024). The liver is the main organ to be affected by AFB_1 but various studies suggest AFB_1 can also induce toxicity in other organs, in both experimental animals and humans. However, few studies have been conducted on aflatoxin-induced alterations in the lungs. In the present study, AFB_1-induced toxicity in lungs was confirmed through biochemical and molecular alterations. In contrast to the damage reported in the current study in the liver and kidney, damage in the lung tissue was less pronounced. The initial injury caused by ROS may lead to localized tissue death, due to which macrophages are activated and cause the release of pro-inflammatory cytokines (Table 3). TNF-α is an important marker in tumor and cancer development. It plays a role in regulating cytokine production through the NF-kB pathway. IL-6 is a pro-inflammatory cytokine associated with innate and adaptive immune response (Zhang et al. 2021). In the present study, AFB_1 exposure significantly increased the TNF-α and IL-6 levels, indicating the activation of systemic inflammation. Treatment with BJE 600 mg/kg b.w. decreased the concentration of pro-inflammatory cytokines, possibly through one of these pathways: mitogen-interacting protein kinases (MAPK), signal transducer and extracellular signal-regulated kinases (ERK). The increase in pro-inflammatory cytokines in the present study was similar to data reportedly previously (Mohammad et al. 2017, Hua et al. 2021, Hatipoglu and Keskin 2022, Salam et al. 2023). It was also observed that AFB_1 selectively increased the caspase-3 gene expression and decreased the expression of the Nrf-2 gene in the liver, kidney and lungs. Under normal conditions, Nrf2 remains bound to Kelch-like ECH-associated protein 1 (Keap1) and is degraded via proteasome. However, during oxidative injury, ROS modify Keap1, preventing Nrf2 degradation (Abed et al. 2015). This allows Nrf2 to enter the nucleus, where it binds to antioxidant response elements (ARE), triggering the expression of detoxification and antioxidant genes. In AFB_1 toxicity, persistent oxidative imbalance leads to Nrf2 degradation, NF-κB suppression and potential epigenetic modifications, collectively impairing the antioxidant defense system (Wang et al. 2021). In contrast to BJE at 300 mg/kg b.w., BJE at 600 mg/kg b.w. strongly enhanced the protein and gene expression of the Nrf2 gene. Western blotting showed increased expression of the active form of caspase-3 protein in AFB_1-treated rats. Caspase-3 is a modulator of apoptosis. In its cleaved form, caspase-3, activates caspase-activated DNase, which is an endonuclease that facilitates the fragmentation of DNA during apoptosis. Therefore, upregulation of caspase-3 and mitochondrial structural damage contribute to enhanced apoptotic signaling in AFB_1-induced toxicity. However, the treatment groups markedly downregulate the expression of caspase-3. The protein levels of cleaved caspase-3 were consistent with the mRNA expression results. The trend of gene expression observed in the present study aligns with findings from previous studies

(Ijaz et al. 2023, Karatekeli et al. 2023, Zhang et al. 2023, Şengül et al. 2024). In conclusion, BJE at 600 mg/kg b.w. significantly mitigated the biochemical, metabolic and oxidative alterations, affirming its strong protective efficacy, comparable to that of silymarin.

Conclusion

This study investigated the protective role of BJE in mitigating the toxic impact of AFB_1 in multiple organs, specifically the lungs, liver and kidneys, of rats. The observed improvements in biochemical, metabolic profiles, antioxidant defense system parameter and regulation of gene expression (Nrf2 and Caspase-3) suggest that BJ has significant antioxidant, anti-inflammatory and cytoprotective potential. These findings highlight the protective potential of BJE as a therapeutic agent in ameliorating AFB_1-induced oxidative and cellular damage in the body. Further research is warranted to identify and quantify the individual bioactive compounds (glucosinolates, flavonoids and phenolic acids) and elucidate the molecular mechanisms underlying the protective effects of BJE and explore its potential clinical relevance.

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