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Piperine Prevents Oxidative Insults, Inflammation, Apoptosis and Histological Alterations Following Aflatoxin B1-induced Hepatotoxicity

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ABSTRACT

Background: Aflatoxin B1 (AFB1), a hepatotoxic mycotoxin produced by Aspergillus species, poses significant health risks through food contamination. Piperine, the bioactive alkaloid from black pepper, exhibits diverse pharmacological properties including hepatoprotection. **Methods:** Forty male Wistar rats were divided into four groups (n = 10): control (saline), AFB1 (100 µg/kg/day), piperine (1.12 mg/kg/day) and a combination group receiving piperine (1.12 mg/kg/day) one hour before AFB1 (100 µg/kg/day) for 28 days. Treatments were administered intraperitoneally for 28 days. Liver function tests, oxidative stress markers, inflammatory cytokines, apoptotic proteins and histopathological changes were evaluated. **Result:** AFB1 exposure significantly elevated liver enzymes (aspartate aminotransferase [AST], alanine aminotransferase [ALT], alkaline phosphatase [ALP]) and decreased albumin levels. Oxidative stress was evident through increased malondialdehyde (MDA) and nitric oxide (NO) levels with concurrent depletion of antioxidants (reduced glutathione [GSH], glutathione peroxidase [GPx], glutathione reductase [GR], superoxide dismutase [SOD], catalase [CAT]) and nuclear factor erythroid 2–related factor 2 (Nrf2) downregulation. Inflammatory responses were marked by elevated interleukin-1 beta (IL-1β), tumor necrosis factor-alpha (TNF-α) and nuclear factor kappa B (NF-κB). AFB1 triggered apoptosis *via* increased caspase-3 and Bcl-2–associated X protein (Bax) with decreased B-cell lymphoma 2 (Bcl-2) expression. Histopathology revealed severe hepatocellular damage including necrosis, vacuolar degeneration and megalocyte formation. Piperine co-treatment significantly attenuated these alterations, restoring liver function parameters, enhancing antioxidant defenses, suppressing inflammatory mediators and normalizing apoptotic markers while preserving hepatic architecture.

Key words: Aflatoxin B1, Apoptosis, Hepatotoxicity, Inflammation, Oxidative stress, Piperine.

INTRODUCTION

Aflatoxin B1 (AFB1), a potent mycotoxin produced by *Aspergillus flavus* and *Aspergillus parasiticus* Mishra *et al.* (2022), represents a significant threat to global food safety and public health (Jallow *et al.*, 2021; Cao *et al.*, 2022). Classified as a Group I carcinogen by the International Agency for Research on Cancer (Humans, 1993), AFB1 is among the most toxic naturally occurring hepatocarcinogens (Wang *et al.*, 2022). Its widespread contamination of agricultural commodities poses substantial challenges to food security worldwide, particularly in regions with suboptimal storage conditions and limited regulatory oversight.

The liver, serving as the primary organ for AFB1 biotransformation and elimination, bears the major toxic burden (Wang *et al.*, 2022). This organ specificity stems from its high expression of cytochrome P450 enzymes responsible for AFB1 activation (Eaton *et al.*, 2025). AFB1-induced hepatotoxicity manifests through multiple pathological mechanisms, including severe hepatic architectural disruption, elevated liver enzymes particularly AST and ALT (El-Bahr, 2015) and extensive cellular damage through necrosis and apoptosis (Ahmed *et al.*, 2022).

Recent research has elucidated critical pathways underlying AFB1's hepatotoxic effects, including oxidative stress, inflammatory responses and mitochondrial dysfunction (Moloi *et al.*, 2024; Albrakati, 2025). Oxidative

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stress emerges as a fundamental mechanism (Lokman *et al.*, 2023), where AFB1 disrupts the delicate balance between cellular oxidants and antioxidants (Liu *et al.*, 2023), leading to extensive damage to cellular biomolecules including DNA, proteins and lipids (Verma, 2004). Concurrently, AFB1 triggers robust inflammatory responses through activation of the NLRP3 inflammasome pathway (Liao *et al.*, 2024; Albrakati, 2025), resulting in enhanced production of pro-inflammatory cytokines including IL-1β and TNF-α (Pan *et al.*, 2024). Furthermore, AFB1 significantly impairs mitochondrial function and disrupts electron transport chain function, leading to increased ROS production and triggering apoptotic pathways (Liao *et al.*, 2024).

In the search for effective hepatoprotective agents against AFB1 toxicity, piperine, a bioactive alkaloid from black pepper (*Piper nigrum*) and long pepper (*Piper longum*), has emerged as a promising candidate (Gorgani *et al.*, 2017). Extensive research demonstrates piperine's remarkable therapeutic potential through anti-inflammatory, antioxidant, antitumor and immunomodulatory activities (Yang *et al.*, 2019; Turrini *et al.*, 2020; Yadav *et al.*, 2023). Its documented ability to enhance natural detoxification pathways and regulate inflammatory responses, coupled with excellent safety profile (NOAEL: 50 mg/kg body weight/day) (Ziegenhagen *et al.*, 2021), provides strong rationale for investigating its potential against AFB1-induced liver injury.

While piperine's hepatoprotective properties are well-documented across various liver injury models, its specific role in protecting against AFB1-induced hepatotoxicity remains incompletely understood. Therefore, this study aims to comprehensively investigate piperine's protective effects against AFB1-induced hepatotoxicity in rats, with particular emphasis on modulation of oxidative stress parameters and antioxidant defense systems, regulation of inflammatory responses and apoptotic signaling pathways.

MATERIALS AND METHODS

Chemicals and reagents

Aflatoxin B1 (AFB1, CAS Number: 1217449-45-0) and piperine (CAS No.: 94-62-2) were purchased from Sigma-Aldrich (St. Louis, MO, USA). All other chemicals and reagents used were of analytical grade.

Animals and experimental design

Male Wistar rats (weighing 180-200 g) were obtained from the King Fahd Center for Genetic Research, King Abdulaziz University, Kingdom of Saudi Arabia. Animals were housed under standard laboratory conditions (temperature 22±2°C, 12-hour light/dark cycle, relative humidity 55±5%) with free access to standard pellet diet and water ad libitum. After one week of acclimatization, rats were randomly divided into four groups (n=10 per group).

- Group I (Control): Rats received intraperitoneal (IP) injection of normal saline for 28 days.
- Group II (AFB1): Rats received IP injection of 100 µg/kg/day of AFB1 for 28 days (Komsky-Elbaz *et al.*, 2018).
- Group III (Piperine): Rats received IP injection of 1.12 mg/kg/day of piperine for 28 days (Dogra *et al.*, 2004).
- Group IV (AFB1 + Piperine): Rats received IP injection of 1.12 mg/kg/day of piperine one hour before AFB1 injection (100 µg/kg/day) for 28 days.

Sample collection and preparation

At the end of the experimental period, rats were fasted overnight and euthanized under light ether anesthesia (Brooks *et al.*, 1999). Blood samples were collected *via* cardiac puncture, allowed to clot and centrifuged at 3000 rpm for 15 minutes to obtain serum. Liver tissues were immediately excised, washed in ice-cold saline and divided into portions for different analyses. Samples for biochemical analyses were stored at -80°C until use.

Biochemical analyses

Liver function tests

Serum levels of aspartate transaminase (AST), alanine transaminase (ALT) and albumin were measured using commercial kits (Biodiagnostic, Egypt).

Oxidative stress index

Malondialdehyde (MDA) was determined through thiobarbituric acid reaction with absorbance measured at 532 nm according to (Ohkawa *et al.,* 1979). Reduced glutathione (GSH) was quantified using Ellman's method with absorbance at 412 nm using the method of Ellman (1959). Nitric oxide (NO) levels were measured using Griess reagent at 540 nm (Robbins *et al.*, 1996). For antioxidant activities, glutathione peroxidase (GPx) was assessed by monitoring NADPH oxidation at 340 nm (Paglia and Valentine, 1967). Glutathione reductase (GR) activity was determined using GSSG and NADPH (Moron *et al.*, 1979). Superoxide dismutase (SOD) activity was evaluated by measuring NBT reduction inhibition at 560 nm (Nishikimi *et al.*, 1972). Catalase (CAT) activity was measured by monitoring H, O, decomposition at 240 nm (Aebi, 1984). Nuclear factor erythroid 2-related factor 2 (Nrf2) levels were determined using ELISA Kit (Elabscience, Cat: E-EL-R1052). All measurements were performed in triplicate.

Inflammatory markers

Levels of interleukin-1β (IL-1β), tumor necrosis factor-α (TNF-α) and nuclear factor kappa B (NF-κB) were measured using commercial ELISA kits (Cat: E-EL-R0012, E-EL-R0019 and E-EL-R0674 respectively, Elabscience Biotechnology Inc., USA).

Apoptotic markers

ELISA kits for Bcl-2 and Bax were used (BioVision, Inc.; catalogue numbers E4513 and CSB-E08854r). Caspase-3 activity was assessed using colorimetric kit (Sigma-Aldrich: CASP3C-1KT).

Histopathological examination

Liver tissue samples were fixed in 10% neutral buffered formalin for 24 hours, processed through ascending grades

of alcohol, cleared in xylene and embedded in paraffin wax. Sections (5 µm thickness) were cut and stained with hematoxylin and eosin (H and E). Stained sections were examined under light microscope (Olympus BX51, Japan) and photographed. Histopathological changes were assessed by a pathologist blinded to experimental groups.

Quantitative-histopathological assessment

Histological alterations including vacuolar degeneration, binucleation and cellular megalocytosis were quantified in ten randomly selected microscopic fields (1 mm^2 each) under 40× magnification (Gibson-Corley *et al.*, 2013; Monmeesil *et al.*, 2019; Ali *et al.*, 2021).

Gene expression analysis

Total RNA was extracted using RNeasy Plus Mini-kit (Qiagen). First-strand cDNA was synthesized using cDNA synthesis kit (Bio-Rad) and amplified using Power SYBR Green (Life Technologies) on Applied Biosystems 7500 instrument. PCR conditions: 95°C for 4 minutes, 40 cycles of 94°C for 60 seconds and 55°C for 60 seconds, final extension at 72°C for 10 minutes. Relative gene expression was determined using comparative Ct method (Livak and Schmittgen, 2001). Primers for Caspase-3 and Nrf2 were designed using Primer-BLAST and synthesized by Jena Bioscience GmbH (Almayouf *et al.*, 2020) (Table 1).

Statistical analysis

Data were expressed as mean±SEM. Statistical analysis was performed using GraphPad Prism 8.0. Differences between groups were analyzed using one-way ANOVA followed by Tukey's post-hoc test. P<0.05 was considered statistically significant.

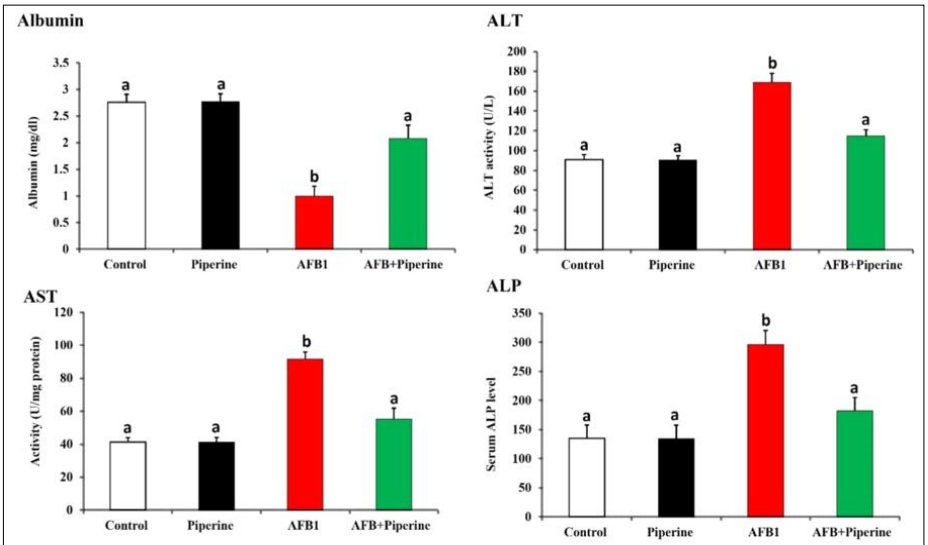
RESULTS AND DISCUSSION

Liver enzyme level tests

The biochemical analysis of liver function parameters revealed significant (P<0.05) implications regarding AFB1-induced hepatotoxicity and piperine's hepatoprotective potential. AFB1 administration induced substantial hepatocellular injury, evidenced by marked elevations in enzymatic markers: AST increased by 130% (92±4 U/mg protein), ALT by 89% (170±8 U/L) and ALP by 127% (295±25 units), accompanied by a severe 64% reduction in albumin synthesis (1.0±0.2 mg/dL). Notably, Piperine co-administration demonstrated significant hepatoprotective efficacy, attenuating enzyme elevations compared to AFB1-treated subjects. Furthermore, piperine intervention preserved protein synthetic capacity, maintaining albumin levels near to the control values. These comprehensive biochemical findings substantiate piperine's multifaceted hepatoprotective mechanisms (Fig 1).

Table 1: Primers sqRT-PCR analysis.

Name	Accession number	Sense (5′-3′)	Antisense (5′-3′)
Nrf2	NM_031789.2	CAGCATGATGGACTTGGAATTG	GCAAGCGACTCATGGTCATC
Caspase-3	NM_012922.2	GGCCGACTTCCTGTATGCTT	CGTACAGTTTCAGCATGGCG
β-actin	NM_031144.3	TCTTCCAGCCTTCCTTCCTG	CACACAGAGTACTTGCGCTC



Values: mean±SD. a,b = Significant differences (p<0.05) vs control and AFB1 groups.

Fig 1: Effects of piperine on serum liver enzymes (ALP, AST, ALT, albumin) in AFB1-induced hepatotoxicity.

Analysis of antioxidant enzyme activities

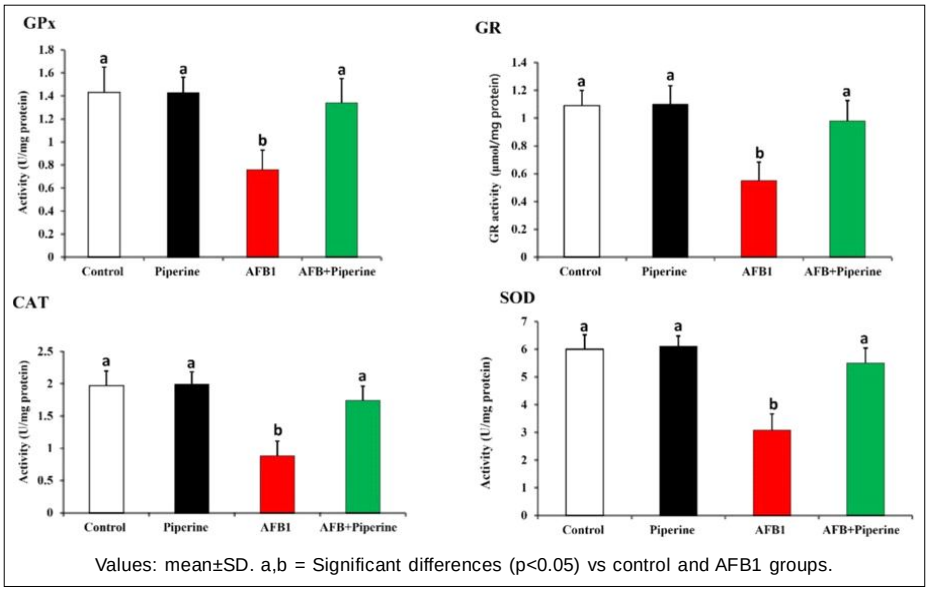
Analysis of antioxidant enzyme activities revealed significant and comprehensive changes across all treatment groups (P<0.05). AFB1 administration induced dramatic reductions across all measured antioxidant enzymes. CAT activity decreased, indicating compromised hydrogen peroxide decomposition. GPx activity reduced, suggesting impaired peroxide neutralization capacity. GR activity showed substantial decline, reflecting disrupted glutathione recycling mechanisms. SOD activity decreased, indicating severely compromised superoxide radical scavenging ability. These coordinated reductions represent comprehensive impairment of cellular antioxidant defense mechanisms.

The combined treatment group (AFB1+Piperine) demonstrated remarkable improvement in all antioxidant enzyme activities. Piperine co-treatment effectively enhanced CAT, GPx, GR and SOD activity compared to the AFB1-treated group. While these enhanced activities remained slightly below control levels, the substantial improvements across all enzymes indicate significant restoration of antioxidant defense capacity (Fig 2).

Analysis of Nrf2, the master regulator of cellular antioxidant responses, demonstrated significant alterations (P<0.05) across treatment groups. AFB1 exposure induced substantial reduction in Nrf2 level and mRNA expression, while piperine co-treatment significantly restored Nrf2 levels, showing marked increase compared to AFB1 treatment, indicating enhanced transcriptional regulation of antioxidant defense mechanisms (Fig 3).

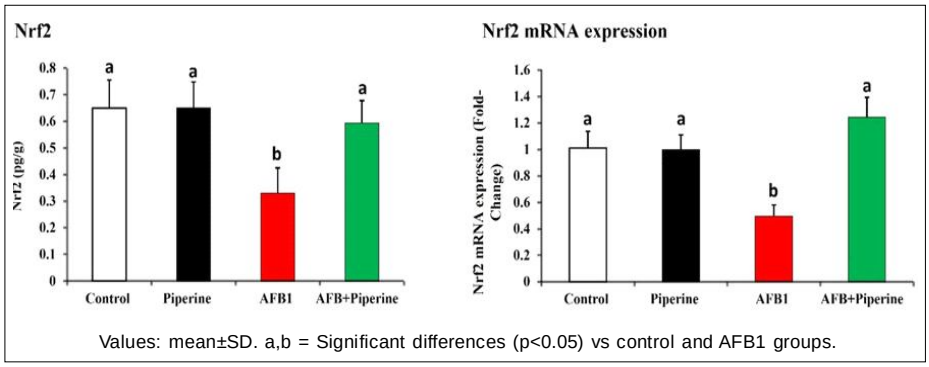
Oxidative stress parameters analysis

The comprehensive analysis of oxidative stress parameters revealed significant alterations (P<0.05) across experimental groups. AFB1 administration induced substantial oxidative stress, manifested through marked elevations in pro-oxidant markers (NO increased and MDA elevated) concurrent with significant depression of antioxidant defense mechanisms (GSH reduced). Notably, piperine co-administration



Values: mean±SD. a,b = Significant differences (p<0.05) vs control and AFB1 groups.

Fig 2: Effects of piperine on hepatic antioxidant enzymes (GPx, GR, CAT, SOD) in AFB1-induced hepatotoxicity.



Values: mean±SD. a,b = Significant differences (p<0.05) vs control and AFB1 groups.

Fig 3: Effects of piperine on Nrf2 level and mRNA expression in AFB1-induced hepatotoxicity.

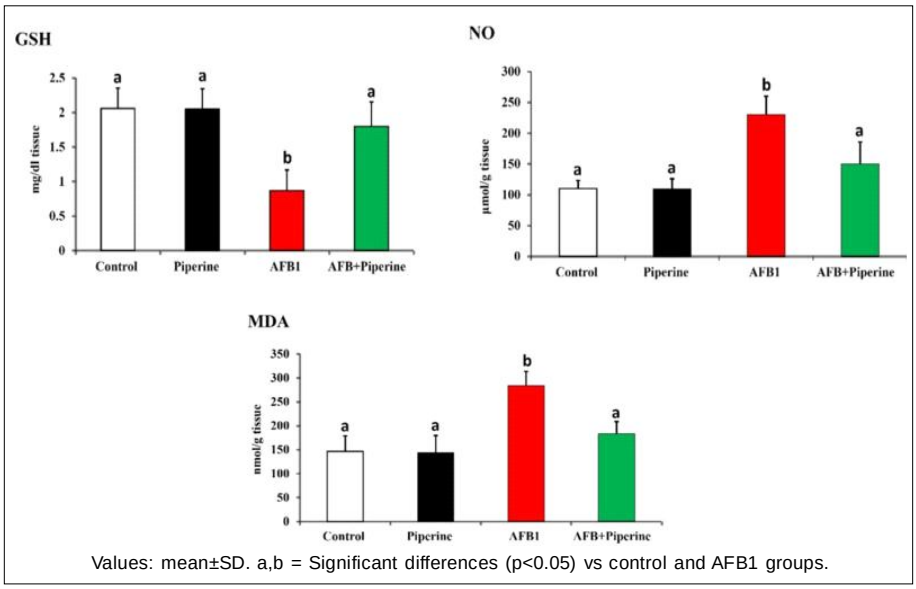
demonstrated significant antioxidant efficacy, attenuating pro-oxidant markers compared to AFB1 group while substantially enhancing antioxidant defense parameters relative to AFB1 treatment (Fig 4).

Analysis of inflammatory markers

The comprehensive analysis of inflammatory markers revealed significant modulation ($P<0.05$) across experimental groups. AFB1 administration induced substantial inflammatory activation, evidenced by marked elevations in pro-inflammatory mediators: IL-1β, TNF-α and NF-κB, collectively indicating comprehensive inflammatory pathway activation. Notably, piperine co-administration exhibited significant anti-inflammatory efficacy, attenuating the elevated inflammatory markers through coordinated reductions in IL-1β, TNF-α and NF-κB compared to AFB1-treated subjects (Fig 5).

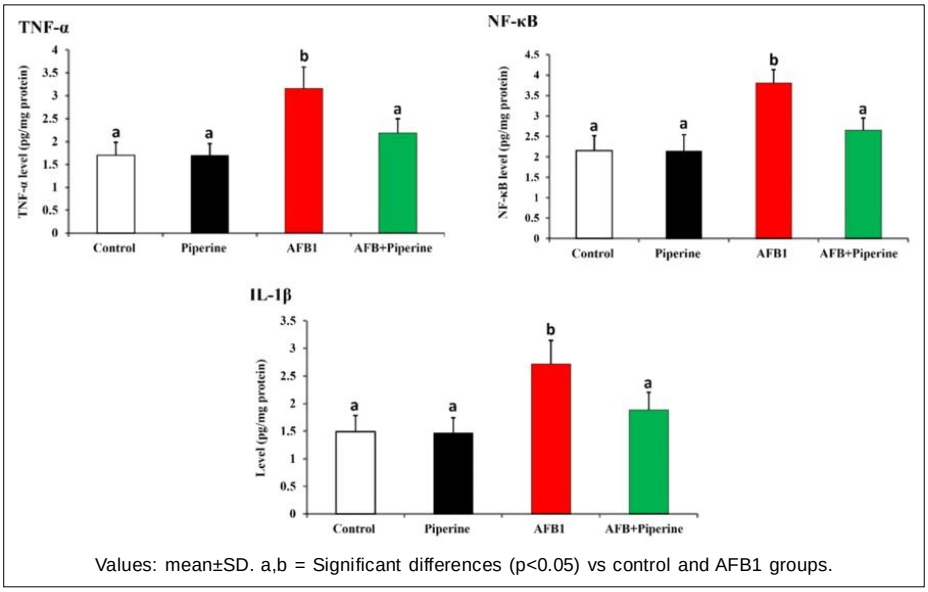
Analysis of apoptotic markers

The study's apoptosis analysis indicated that expression of anti-apoptotic protein Bcl-2 was significantly decreased ($p<0.05$) in the AFB1 group compared to the control. However, both the piperine group and the AFB1+piperine group maintained Bcl-2 levels similar to the control. For pro-apoptotic protein Bax, the AFB1 group showed significantly increased levels compared to the control.



Values: mean±SD. a,b = Significant differences ($p<0.05$) vs control and AFB1 groups.

Fig 4: Effects of piperine on oxidative stress markers (GSH, NO, MDA) in AFB1-induced hepatotoxicity.



Values: mean±SD. a,b = Significant differences ($p<0.05$) vs control and AFB1 groups.

Fig 5: Effects of piperine on inflammatory markers (TNF-α, IL-1β, NF-κB) in AFB1-induced hepatotoxicity.

Treatment with piperine alone maintained Bax levels comparable to control values, while combination treatment (AFB1 + piperine) effectively reduced elevated Bax levels induced by AFB1 exposure. Caspase-3 exhibited significant elevation in the AFB1 group compared to the control. This increase was observed at both protein and mRNA expression levels. Both the piperine group and the AFB1+piperine group maintained caspase-3 protein and mRNA expression levels similar to control values (Fig 6).

Histological analysis

Histological examination revealed distinct histopathological changes across experimental groups. Control group exhibited normal hepatic architecture with well-organized hexagonal lobules. Treatment with piperine alone maintained normal liver histology. AFB1 administration induced severe alterations including central vein dilatation and congestion, apoptotic hepatocytes, focal hepatocellular necrosis with Kupffer cell proliferation, interlobular vein distention, vacuolar degeneration and megalocyte presence. Co-treatment with piperine ameliorated these histopathological changes, showing restored tissue architecture, reduced cellular degeneration, normalized sinusoidal spaces and presence of binucleated hepatocytes indicating active cellular regeneration (Fig 7).

Histomorphological alterations in hepatic tissue

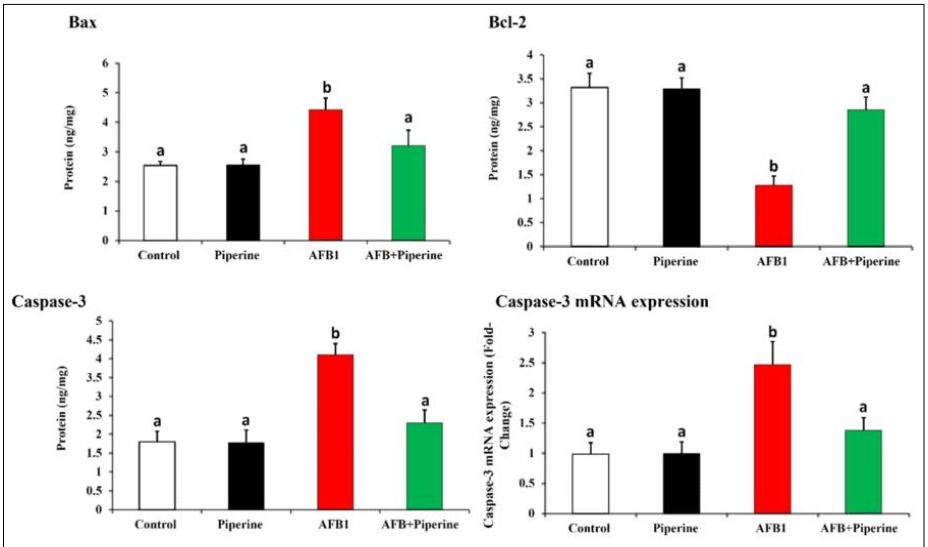
Comprehensive histopathological assessment revealed AFB1 induced severe vacuolar degeneration (2.7 cells/mm^2) and megalocyte formation (1.7 cells/mm^2), representing 4-fold and 3.4-fold increases respectively compared to control values. Piperine pre-treatment demonstrated substantial hepatoprotective efficacy through 59% reduction in both vacuolar degeneration (1.1 cells/mm^2) and megalocyte prevalence (0.7 cells/mm^2). Concurrently, AFB1+Piperine intervention exhibited enhanced binucleated hepatocyte formation (2.9 cells/mm^2), representing 53% increase compared to AFB1 administration alone (Fig 8).

The present study provides comprehensive evidence for the hepatoprotective effects of piperine against AFB1-induced liver injury through multiple mechanisms, including modulation of oxidative stress, inflammation and apoptotic pathways. The results demonstrate significant improvements in liver function parameters, antioxidant defense systems and inflammatory markers upon piperine co-treatment with AFB1.

The biochemical analysis revealed significant insights into AFB1-induced hepatotoxicity and piperine's hepatoprotective mechanisms. AFB1 administration induced substantial hepatocellular injury, evidenced by marked elevations in AST, ALT and ALP, alongside a severe reduction in albumin synthesis. These results are consistent with the findings of Yan *et al.* (2022) and Rotimi *et al.* (2017), who documented analogous patterns of hepatic enzyme dysregulation. Piperine exhibited notable hepatoprotective effects, diminishing AST, ALT and ALP levels in comparison to AFB1-treated subjects.

The intervention also preserved protein synthetic function. These results indicate membrane stabilization, biliary function preservation and protein synthesis maintenance mechanisms, consistent with recent studies while providing more comprehensive quantification of protective parameters.

The comprehensive biochemical investigation of oxidative stress parameters reveals intricate insights into the molecular pathways underlying AFB1-induced hepatotoxicity and piperine's protective mechanisms. The experimental findings demonstrate significant perturbations in cellular redox homeostasis following AFB1



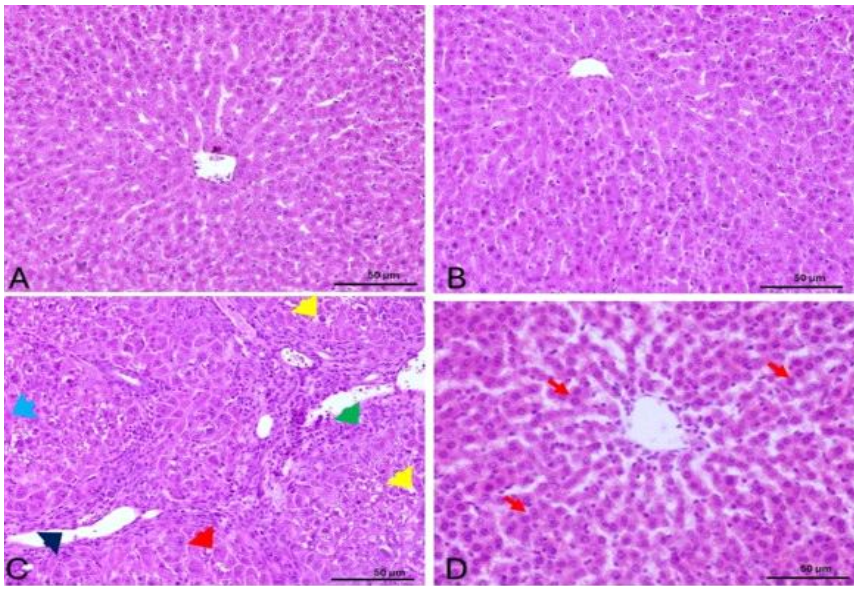
Values: mean ± SD. a,b = Significant differences ($p<0.05$) vs control and AFB1 groups.

Fig 6: Effects of piperine on apoptotic markers (Bax, Bcl-2, Caspase-3) in AFB1-induced hepatotoxicity.

exposure, characterized by substantial elevations in pro-oxidant markers (NO and MDA), indicating severe oxidative damage and membrane lipid peroxidation. These alterations align with recent findings by Rotimi *et al.* (2019), who documented similar patterns of oxidative stress in hepatic tissue following AFB1 exposure.

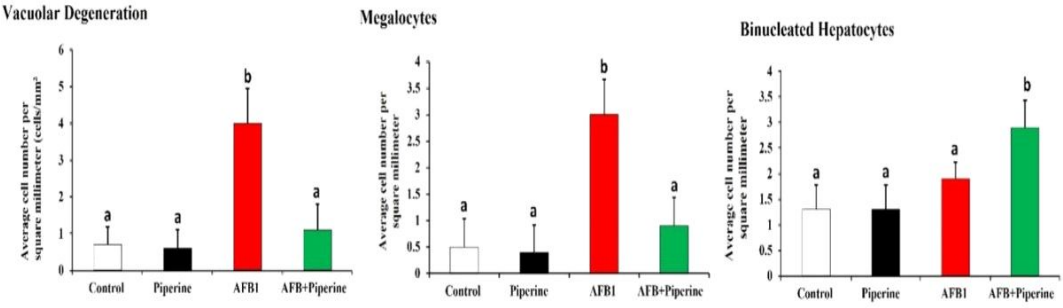
The molecular cascade of oxidative damage appears to operate through multiple interconnected pathways. The significant increase in NO indicates the upregulation of inducible nitric oxide synthase (iNOS), which results in the formation of peroxynitrite, subsequent protein nitrosylation and mitochondrial dysfunction. Concurrently, the substantial increase in MDA indicates extensive membrane phospholipid peroxidation, resulting in structural compromise and cellular dysfunction. These results build on earlier work by Li *et al.* (2022), who found similar ways that AFB1 can damage membranes in the liver.

The analysis of antioxidant defense parameters revealed comprehensive impairment across multiple protective systems. The significant depletion of reduced glutathione indicates severely compromised cellular redox buffering capacity and detoxification potential. This disruption of glutathione homeostasis corresponds with observations by Kövesi *et al.* (2020). The coordinated reduction in antioxidant enzyme activities (CAT, GPx, GR, SOD) demonstrates systematic impairment of cellular defense mechanisms, compromising hydrogen peroxide



(A) Control- Normal architecture; (B) Piperine- Normal structure; (C) AFB1- Severe damage with central vein dilatation (black arrow), apoptotic hepatocytes (blue), focal necrosis (green), interlobular vein distention (purple), vacuolar degeneration (yellow), megalocytes (red); (D) AFB1+Piperine - restored architecture with binucleated hepatocytes (red). Scale bar = 50 µm.

Fig 7: H and E-stained liver sections.



Values: Mean±SD. a,b = Significant differences ($p<0.05$) vs control and AFB1 groups.

Fig 8: Effects of piperine on hepatocellular alterations (Vacuolar degeneration, megalocytes, binucleated hepatocytes) in AFB1-induced hepatotoxicity.

decomposition, peroxide neutralization, glutathione recycling and superoxide radical scavenging capabilities.

Notably, piperine intervention demonstrated remarkable protective efficacy through multiple mechanisms. The compound significantly attenuated pro-oxidant markers while substantially enhancing antioxidant defense parameters. The restoration of antioxidant enzyme activities suggests comprehensive enhancement of cellular defense capabilities. These protective effects appear to be mediated through modulation of the Nrf2 pathway, as evidenced by the increase in Nrf2 levels and mRNA expression compared to AFB1 treatment, indicating enhanced transcriptional regulation of antioxidant defense mechanisms. This multi-targeted approach provides more comprehensive protection compared to previous studies focusing on isolated antioxidant mechanisms.

Our comparative analysis of inflammatory markers reveals significant concordance with previous research regarding AFB1-induced inflammatory responses and piperine's therapeutic intervention. The experimental findings indicate that AFB1 exposure induces a significant activation of the inflammatory cascade, as evidenced by pronounced increases in critical inflammatory mediators. Our observations of increased IL-1β and TNF-α closely parallel the findings of Li *et al.* (2022). Similarly, the observed increase in NF-κB activation aligns with Huang *et al.* (2019), further validating the inflammatory profile associated with AFB1 toxicity.

The therapeutic efficacy of piperine demonstrated in our study both confirms and extends previous research findings. Our observed reductions in inflammatory markers closely correspond with reported attenuations. Nonetheless, our study offers innovative insights by illustrating NF-κB suppression, indicating a fundamental mechanism by which piperine influences inflammatory signaling pathways. This modulation of NF-κB activity represents a critical advancement in understanding piperine's therapeutic mechanism, indicating its capacity to regulate inflammatory responses at the transcriptional level.

The analysis of apoptotic markers reveals sophisticated regulatory mechanisms governing cellular survival pathways. The experimental findings revealed significant perturbations in apoptotic cascade regulation following AFB1 exposure. The observed elevation in Caspase-3 and Bax levels indicates substantial activation of executing apoptotic machinery. This elevation occurred concurrently with marked reductions in anti-apoptotic Bcl-2, suggesting comprehensive dysregulation of mitochondrial-mediated death pathways. Research by Muhmood *et al.* (2024) documented similar patterns of apoptotic dysregulation in AFB1-induced hepatotoxicity. Piperine intervention demonstrated remarkable efficacy in normalizing apoptotic parameters through multi-targeted mechanisms, significantly attenuating elevated Caspase-3 and Bax levels while concurrently restoring Bcl-2 towards physiological levels.

Histological analysis revealed critical morphological alterations supporting the biochemical and molecular evidence. Exposure to AFB1 resulted in pronounced hepatotoxic effects, characterized by central vein dilation and vascular congestion, hepatocyte apoptosis, focal areas of hepatocellular necrosis accompanied by Kupffer cell proliferation, prominent vacuolar degeneration and the emergence of megalocytes findings that are consistent with Millimouno *et al.* (2014). Co-administration of piperine significantly attenuated these pathological changes, restoring hepatic architecture, reducing cellular degeneration and normalizing sinusoidal spaces. The observed histological recovery suggests that piperine mitigates AFB1-induced hepatotoxicity not only by modulating xenobiotic metabolism but also by enhancing hepatocellular regeneration.

CONCLUSION

This study demonstrates piperine's significant hepatoprotective potential against AFB1-induced liver injury through multiple mechanisms. Piperine ameliorates hepatotoxicity by preserving liver function through attenuation of elevated liver enzymes and maintenance of serum albumin; enhancing antioxidant defenses via Nrf2 pathway activation and oxidative stress reduction; modulating inflammatory response through NF-êB pathway inhibition; regulating apoptotic signaling via Caspase-3 and Bcl-2/Bax modulation; and protecting hepatic tissue architecture. These findings establish piperine as a promising therapeutic agent against AFB1-induced hepatotoxicity, warranting further research to optimize dosing strategies and evaluate clinical safety and efficacy.

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Authors' contribution

Xiuwen Li, Jing Zhao and Shadi Tamur contributed to data analysis, literature review, writing, visualisation and draft preparation. Naif Alsiwiehri also contributed to data curation. Ashraf Albrakati and AI assisted in data collection. Ashraf Albrakati and Naif Alsiwiehri were responsible for animal handling. Tamer Ali Sweellum and Mahmoud H. Khedr contributed to the manuscript's conceptualisation, validation, data curation and editing. Ashraf Albrakati, Shadi Tamur and Mohamed A. Elhefny

further contributed to the validation and interpretation of results.

Ethical approval

All experimental procedures were conducted in accordance with the Committee of Research Ethics for Laboratory Animal Care at the Anatomy Department, School of Medicine, Taif University (approval number: HAO-02-T-105).

Declaration of conflicting interests

The authors declared no potential conflicts of interest with respect to the research, authorship and/or publication of this article.

REFERENCES

Aebi, H. (1984). [13] Catalase *in vitro*. In: Methods in Enzymology. *Elsevier.* pp. 121-126.

Ahmed, N., El-Rayes, S.M., Khalil, W.F., Abdeen, A., Abdelkader, A., Youssef, M., Maher, Z.M., Ibrahim, A.N., Abdelrahman, S.M. and Ibrahim, S.F. (2022). Arabic gum could alleviate the aflatoxin b1-provoked hepatic injury in rat: The involvement of oxidative stress, inflammatory and apoptotic pathways. *Toxins.* **14(9):** 605.

Albrakati, A. (2025). Evaluation of the hepatoprotective role of diosmin in a rat model of thioacetamide-induced liver fibrosis. *Indian Journal of Animal Research*. **59(10):** 1681-1689. doi: 10.18805/IJAR.BF-2010.

Albrakati, A. (2025). Protective effects of crocetin against thioacetamide-induced liver fibrosis in rats. *Indian Journal of Animal Research.* **59(9):** 1521-1529. doi: 10.18805/IJAR.BF-1976.

Ali, F.A.Z., Abdel-Maksoud, F.M., Abd Elaziz, H.O., Al-Brakati, A. and Elmahallawy, E.K. (2021). Descriptive histopathological and ultrastructural study of hepatocellular alterations induced by aflatoxin b1 in rats. *Animals.* **11(2):** 509.

Almayouf, M.A., El-Khadragy, M.F., Awad, M.A. and Al-Olayan, E.M. (2020). The effects of silver nanoparticles biosynthesized using fig and olive extracts on cutaneous leishmaniasis induced inflammation in female balb/c mice. *Biosci Rep.* **40(12):** BSR20202672. doi: 10.1042/bsr20202672.

Brooks, S.P., Lampi, B.J. and Bihun, C.G. (1999). The influence of euthanasia methods on rat liver metabolism. *Journal of the American Association for Laboratory Animal Science.* **38(6):** 19-24.

Cao, W., Yu, P., Yang, K. and Cao, D. (2022). Aflatoxin b1: Metabolism, toxicology and its involvement in oxidative stress and cancer development. *Toxicology Mechanisms and Methods.* **32(6):** 395-419.

Dogra, R.K., Khanna, S. and Shanker, R. (2004). Immunotoxicological effects of piperine in mice. *Toxicology.* **196(3):** 229-236.

Eaton, D.L., Williams, D.E. and Coulombe, R.A. (2025). Species differences in the biotransformation of aflatoxin b1: Primary determinants of relative carcinogenic potency in different animal species. *Toxins*. **17(1):** 30. Available from https://www.mdpi.com/2072-6651/17/1/30.

El-Bahr, S. (2015). Effect of curcumin on hepatic antioxidant enzymes activities and gene expressions in rats intoxicated with aflatoxin b1. *Phytotherapy Research.* **29(1):** 134-140.

Ellman, G.L. (1959). Tissue sulfhydryl groups. *Archives of Biochemistry and Biophysics.* **82(1):** 70-77.

Gibson-Corley, K.N., Olivier, A.K. and Meyerholz, D.K. (2013). Principles for valid histopathologic scoring in research. *Veterinary Pathology.* **50(6):** 1007-1015.

Gorgani, L., Mohammadi, M., Najafpour, G.D. and Nikzad, M. (2017). Piperine-the bioactive compound of black pepper: From isolation to medicinal formulations. Comprehensive Reviews in Food Science and Food Safety. **16(1):** 124-140.

Huang, L., Zhao, Z., Duan, C., Wang, C., Zhao, Y., Yang, G., Gao, L., Niu, C., Xu, J. and Li, S. (2019). Lactobacillus plantarum c88 protects against aflatoxin b 1-induced liver injury in mice *via* inhibition of nf-κb-mediated inflammatory responses and excessive apoptosis. *BMC Microbiology.* **19:** 1-9.

Humans, I.W.G.O.T.E.O.C.R.T. (1993). Aflatoxins. In: Some naturally occurring substances: Food items and constituents, heterocyclic aromatic amines and mycotoxins. International Agency for Research on Cancer.

Jallow, A., Xie, H., Tang, X., Qi, Z. and Li, P. (2021). Worldwide aflatoxin contamination of agricultural products and foods: From occurrence to control. *Comprehensive Reviews in Food Science and Food Safety.* **20(3):** 2332-2381.

Komsky-Elbaz, A., Saktsier, M. and Roth, Z. (2018). Aflatoxin b1 impairs sperm quality and fertilization competence. *Toxicology*. **393:** 42-50.

Kövesi, B., Cserháti, M., Erdélyi, M., Zándoki, E., Mézes, M. and Balogh, K. (2020). Lack of dose-and time-dependent effects of aflatoxin b1 on gene expression and enzymes associated with lipid peroxidation and the glutathione redox system in chicken. *Toxins.* **12(2):** 84.

Li, C., Liu, X., Wu, J., Ji, X. and Xu, Q. (2022). Research progress in toxicological effects and mechanism of aflatoxin b1 toxin. *Peer J.* **10:** e13850.

Li, S., Liu, R., Xia, S., Wei, G., Ishfaq, M., Zhang, Y. and Zhang, X. (2022). Protective role of curcumin on aflatoxin b1-induced tlr4/ripk pathway mediated-necroptosis and inflammation in chicken liver. *Ecotoxicology and Environmental Safety*. **233:** 113319.

Liao, C., Xu, F., Yu, Z., Ding, K. and Jia, Y. (2024). The novel role of the nlrp3 inflammasome in mycotoxin-induced toxicological mechanisms. *Vet Sci.* **11(7):** 291. doi: 10.3390/vetsci 11070291.

Liu, Y., Hao, C., Li, L., Zhang, H., Zha, W., Ma, L., Chen, L. and Gan, J. (2023). The role of oxidative stress in the development and therapeutic intervention of hepatocellular carcinoma. *Current Cancer Drug Targets.* **23(10):** 792-804.

Livak, K.J. and Schmittgen, T.D. (2001). Analysis of relative gene expression data using real-time quantitative pcr and the 2(-delta delta c(t)) method. *Methods*. **25(4):** 402-408. doi: 10.1006/meth.2001.1262.

Lokman, M.S., Althagafi, H.A., Alharthi, F., Habotta, O.A., Hassan, A.A., Elhefny, M.A. and Al Sberi, H. *et al.* (2023). Protective effect of quercetin against 5-fluorouracil-induced cardiac impairments through activating nrf2 and inhibiting nf-κb and caspase-3 activities. *Environ. Sci. Pollut. Res. Int.* **30(7):** 17657-17669. doi: 10.1007/s11356-022-23314-z.

Millimouno, F.M., Dong, J., Yang, L., Li, J. and Li, X. (2014). Targeting apoptosis pathways in cancer and perspectives with natural compounds from mother nature. *Cancer Prevention Research.* **7(11):** 1081-1107.

Mishra, S., Jena, C., Naik, P., Kumar, D., Swain, B., Panda, S., Pamia, J. and Jena, G. (2022). Impact of dietary aflatoxin-b1 on juvenile growth in white pekin ducks. *Indian Journal of Animal Research.* **56(2):** 175-181. doi: 10.18805/IJAR.B-4556.

Moloi, T.P., Ziqubu, K., Mazibuko-Mbeje, S.E., Mabaso, N.H. and Ndlovu, Z. (2024). Aflatoxin b1-induced hepatotoxicity through mitochondrial dysfunction, oxidative stress and inflammation as central pathological mechanisms: A review of experimental evidence. *Toxicology*. pp. 153983.

Monmeesil, P., Fungfuang, W., Tulayakul, P. and Pongchairerk, U. (2019). The effects of astaxanthin on liver histopathology and expression of superoxide dismutase in rat aflatoxicosis. *Journal of Veterinary Medical Science.* **81(8):** 1162-1172.

Moron, M.S., Depierre, J.W. and Mannervik, B. (1979). Levels of glutathione, glutathione reductase and glutathione s-transferase activities in rat lung and liver. *Biochimica et Biophysica Acta (BBA)-General Subjects*. **582(1):** 67-78.

Muhmood, A., Liu, J., Liu, D., Liu, S., Azzam, M.M., Junaid, M.B., Hou, L., Le, G. and Huang, K. (2024). Mitigation of deoxynivalenol (don)-and aflatoxin b1 (afb1)-induced immune dysfunction and apoptosis in mouse spleen by curcumin. *Toxins*. **16(8):** 356.

Nishikimi, M., Rao, N.A. and Yagi, K. (1972). The occurrence of superoxide anion in the reaction of reduced phenazine methosulfate and molecular oxygen. *Biochemical and Biophysical Research Communications.* **46(2):** 849-854.

Ohkawa, H., Ohishi, N. and Yagi, K. (1979). Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Analytical Biochemistry*. **95(2):** 351-358.

Paglia, D.E. and Valentine, W.N. (1967). Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. *The Journal of Laboratory and Clinical Medicine.* **70(1):** 158-169.

Pan, H., Hu, T., He, Y., Zhong, G., Wu, S., Jiang, X., Rao, G., You, Y., Ruan, Z., Tang, Z. and Hu, L. (2024). Curcumin attenuates aflatoxin b1-induced ileum injury in ducks by inhibiting nlrp3 inflammasome and regulating tlr4/nf-κb signaling pathway. *Mycotoxin Res.* **40(2):** 255-268. doi: 10.1007/s12550-024-00524-7.

Robbins, R.A., Floreani, A.A., Von Essen, S.G., Sisson, J.H., Hill, G.E., Rubinstein, I. and Townley, R.G. (1996). Measurement of exhaled nitric oxide by three different techniques. *American Journal of Respiratory and Critical Care Medicine.* **153(5):** 1631-1635.

Rotimi, O.A., Rotimi, S.O., Duru, C.U., Ebebeinwe, O.J., Abiodun, A.O., Oyeniyi, B.O. and Faduyile, F.A. (2017). Acute aflatoxin b1-induced hepatotoxicity alters gene expression and disrupts lipid and lipoprotein metabolism in rats. *Toxicology Reports.* **4:** 408-414.

Rotimi, O.A., Rotimi, S.O., Goodrich, J.M., Adelani, I.B., Agbonihale, E. and Talabi, G. (2019). Time-course effects of acute aflatoxin b1 exposure on hepatic mitochondrial lipids and oxidative stress in rats. *Frontiers in Pharmacology*. **10:** 467.

Turrini, E., Sestili, P. and Fimognari, C. (2020). Overview of the anticancer potential of the “king of spices” piper nigrum and its main constituent piperine. *Toxins.* **12(12):** 747.

Verma, R. (2004). Aflatoxin cause DNA damage. *International Journal of Human Genetics.* **4(4):** 231-236.

Wang, L., Huang, Q., Wu, J., Wu, W., Jiang, J., Yan, H., Huang, J., Sun, Y. and Deng, Y. (2022). The metabolism and biotransformation of afb1: Key enzymes and pathways. *Biochemical Pharmacology.* **199:** 115005.

Wang, W., Liu, Q., Liang, X., Kang, Q. and Wang, Z. (2022). Protective role of naringin loaded solid nanoparticles against aflatoxin b1 induced hepatocellular carcinoma. *Chemico-Biological Interactions*. **351:** 109711.

Yadav, S.S., Singh, M.K., Hussain, S., Dwivedi, P., Khattri, S. and Singh, K. (2023). Therapeutic spectrum of piperine for clinical practice: A scoping review. *Critical Reviews in Food Science and Nutrition*. **63(22):** 5813-5840.

Yan, J., Chen, L., Zhang, L., Zhang, Z., Zhao, Y., Wang, Y. and Ou, J. (2022). New insights into the persistent effects of acute exposure to afb1 on rat liver. *Frontiers in Microbiology.* **13:** 911757.

Yang, J.Y., Zhang, J. and Zhou, G. (2019). Black pepper and its bioactive constituent piperine: Promising therapeutic strategies for oral lichen planus. *Inflammopharmacology.* **27:** 5-13.

Ziegenhagen, R., Heimberg, K., Lampen, A. and Hirsch-Ernst, K.I. (2021). Safety aspects of the use of isolated piperine ingested as a bolus. *Foods*. **10(9).** doi: 10.3390/foods 10092121.