Therapeutic and antioxidant effects of lactoperoxidase on aflatoxin B1-induced nephrotoxicity in adult male rats

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Background and objective
Aflatoxin B1 (AFB1), a type of mycotoxin, is present in food and feed and is toxic to both people and animals. Histological effects of AFB1 on the rat kidney have not been well understood. The objective of this study was to evaluate the therapeutic effect of lactoperoxidase (LPO) against aflatoxin B1-induced nephrotoxicity in a trial to improve its clinical use.
Materials and methods
Adult male Wistar rats (150–200 g b.w) were randomly divided into four groups (10 rats each): (1) healthy control group, (2) healthy rats treated IP with LPO (50 mg/kg/day) for 6 weeks, (3) rats intoxicated orally with AFB1 (80 μg/kg/day) for 6 weeks, and (4) Animals treated with LPO for 6 weeks after intoxication with AFB1.
Results and conclusion
The results showed that LPO was successful in reducing aflatoxin B1-induced nephrotoxicity after 6 weeks of treatment. This was demonstrated by the significant decrease in blood urea, urea nitrogen, creatinine, uric acid, potassium, magnesium, phosphorus, TNF-α, IL-1β, as well as kidney NO, MDA, and DNA damages matched with a significant increase in CD4 and albumin levels as well as kidney GSH and SOD. Furthermore, the LPO was successful in aflatoxin B1-induced tissue degenerations, reflecting its therapeutic potential. In conclusion, due to their antioxidant and radical scavenging properties, LPO may be as effective in improving nephrons from aflatoxin B1-induced nephrotoxicity.

Keywords:
aflatoxin B1, Immunomodulation, lactoperoxidase, nephrotoxicity, rat

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Introduction

Aspergillus flavus and *Aspergillus parasiticus*, two filamentous fungi, produce aflatoxins as mycotoxins [1]. They are common environmental pollutants that are also present in a lot of human meals and animal feeds. Aflatoxin B1 (AFB1), one of the numerous known mycotoxins, is far more dangerous than organic pesticides, arsenic, and cyanide, and is the most poisonous, carcinogenic, and teratogenic [2,3]. Approximately 300 parts per billion (ppb) and parts per billion (ppb) have been reported as the acceptable limits for AFB1 in human diet and animal feed, respectively [4]. Growth retardation, illnesses of the digestive and reproductive systems, tissue toxicity, neurotoxicity, and immunosuppression are a few of the major health problems that can result from a high incidence of AFB1 and its accumulation in people and animals through the food chain [5–7].

It is noteworthy that in addition to the liver, the kidney is also a potential target organ for AFB1 [8]. High quantities of poisonous chemicals are also deposited in the renal medulla because drug or toxic substance metabolites are selectively taken up and concentrated by renal tubular cells before being eliminated in the urine [9,10]. This implies that the kidney may also contribute to the buildup of the toxin AFB1. According to research, oxidative stress is a significant risk factor for AFB1 toxicity. Exposure to AFB1 increases reactive oxygen species (ROS) levels, which can harm cellular redox balance and result in kidney damage from oxidative stress [11]. Therefore, it is emphasized that reducing oxidative stress is a successful method of treating AFB1 nephrotoxicity.

An enzyme called lactoperoxidase (LPO) is secreted, among other things, into the saliva, milk, and other bodily fluids. LPO takes part in an all-purpose humoral immune response that targets bacteria, fungi, and viruses in mucosal membranes [12]. Together with hydrogen peroxide, thiocyanate ions, iodides, or bromides, LPO creates the LPO system. This system works by using hydrogen peroxide to oxidize thiocyanate ions (also known as iodides and bromides) into hypothiocyanite ions (also known as hypoiodides

and hypobromides). The function of the thiol groups in the amino acid residues of microbial proteins is oxidized by hypothiocyanite ions, which inhibit the cell division or cause the bacterium to die [13].

The LPO system is one of the primary anticaries defensive systems in the context of the oral cavity [14], additionally, controlling the microflora's makeup and stopping the development of harmful germs linked to periodontitis [15]. Through the development of oral hygiene products supplemented with the LPO system, the physiological characteristics of the enzyme have been utilized in the prevention of various disorders. Bovine milk, which has undergone a well-defined purification process, is the enzyme's industrial source. Its structural and functional properties are strikingly comparable to those of human lactoperoxidase [16]. Consequently, the current study's objective was to investigate any potential modulatory effect of LPO against kidney damage and oxidative stress brought on by aflatoxin B1.

Materials and methods

Aflatoxin B1 (AFB1) CAS number:1162-65-8, molecular weight: 312.27, Beilstein: 1269174, EC number: 214-603-3, MDL number: MFCD00869647, PubChem Substance ID:24891116 and lactoperoxidase (LPO) CAS number 9003-99-0, enzyme commission 1.11.1.7, EC232-668-6, MDLMFCD00131484, and/or NACRES numbers NA.22. AFB1 and LPO were obtained from Sigma Aldrich (St. Louis, MO, USA).

Animals and experimental design

From the Animal Colony, National Research Center Egypt, 40 adult male albino rats weighing between 150 and 200 g were obtained. For 1 week before the start of the experiment, the animals were kept at a temperature of 25°C and light-controlled (12/12 h light/dark cycle) environments with free access to food and water; according to the protocols approved (AZHAR, 15/2022) by the Ethics Committee of the Faculty of Science, Al-Azhar University, Assuit, Egypt. The animals got human care in accordance with the institution's normal criteria. Following acclimation to the conditions in the experimental room, the animals were randomly separated into four groups, each with 10 rats: group (1) normal control rats orally received 0.5 mL/kg/day water for consecutive 6 weeks, group (2) rats IP treated with LPO (50 mg/kg/day) for 6 weeks, group (3) rats orally intoxicated with AFB1 (80μ g/kg/day) for 6 weeks [17], and group (4) rats treated intraperitoneally with LPO for 6 weeks after intoxicated with AFB1 at the mentioned doses and durations.

Blood and tissue sampling

Following an overnight fast and anesthesia rats were intramuscularly injected (IM) with sodium pentobarbital (9.1 mg/kg). All animals were weighed at the end of the treatment period. Heparinized, sterile glass capillaries were used to take blood samples from the retro-orbital plexus. The blood samples were cool-centrifuged at 3000 rpm for 10 minutes, and then the sera were isolated from the blood samples, divided into aliquots, and kept at −80°C until biochemical tests could be made. After blood collection, the animals were quickly decapitated, and a portion of each kidney was removed, washed in saline, dried, rolled in a piece of aluminum foil, and stored at −80°C for biochemical and DNA fragmentation examinations. Another part of the kidney was soaked in a formalin-saline (10%) buffer for histopathological processing and microscopic examination.

Tissue homogenization

A kidney sample was homogenized in an ice-cold phosphate buffer (50 mM, pH 7.4) to obtain a 10% homogenate (w/v). The homogenate was then centrifuged at 5000 rpm for 20 minutes to separate the nuclear and mitochondrial fractions. After that, the supernatant of each sample was then divided into aliquots and stored at −80°C until the biochemical measurements could be assessed.

Biochemical determinations

Serum urea, urea nitrogen, creatinine, uric acid, albumin, calcium, sodium, magnesium, phosphorus, and potassium levels were measured using reagent kits bought from DiaSys Diagnostic System GmbH, Germany, and spectrophotometry. Using reagent kits purchased from Biodiagnostic, Giza, Egypt, kidney levels of reduced glutathione (GSH), nitric oxide (NO), as well as activity of superoxide dismutase (SOD) and catalase (CAT) were measured. The level of malondialdehyde (MDA) was determined chemically as described by Ruiz-Larnea *et al.* [18].

Proinflammatory cytokines

ELISA was used to measure serum levels of tumor necrosis factor-alpha (TNF-α), interleukin-1beta (IL-1β), and CD4 using reagent kits from SinoGeneClon Biotech Co., Ltd., No. 9 BoYuan Road, YuHang District 311112, Hang Zhou, China.

Kidney DNA fragmentation percentage

The percentage of kidney DNA fragmentation was determined by separating the cleaved DNA from the intact chromatin by centrifugation and measuring the amount of DNA present in the supernatant and pellet using the diphenylamine assay according to

the quantitative method used for grading DNA damage [19]. The degree of DNA fragmentation refers to the ratio of DNA in the supernatant to the total DNA in the supernatant and pellet, and the proportion of fragmented DNA was calculated from the absorbance reading at 578 nm using the following equation:

$$\text{DNA fragmentation}\% = \frac{\text{A supernatant}}{\text{A supernatant} + \text{A pellet}}$$

Histopathology

Hematoxylin and Eosin [20] was used to stain paraffin sections (5 µ thick), which were examined under a light microscope.

Statistical analysis

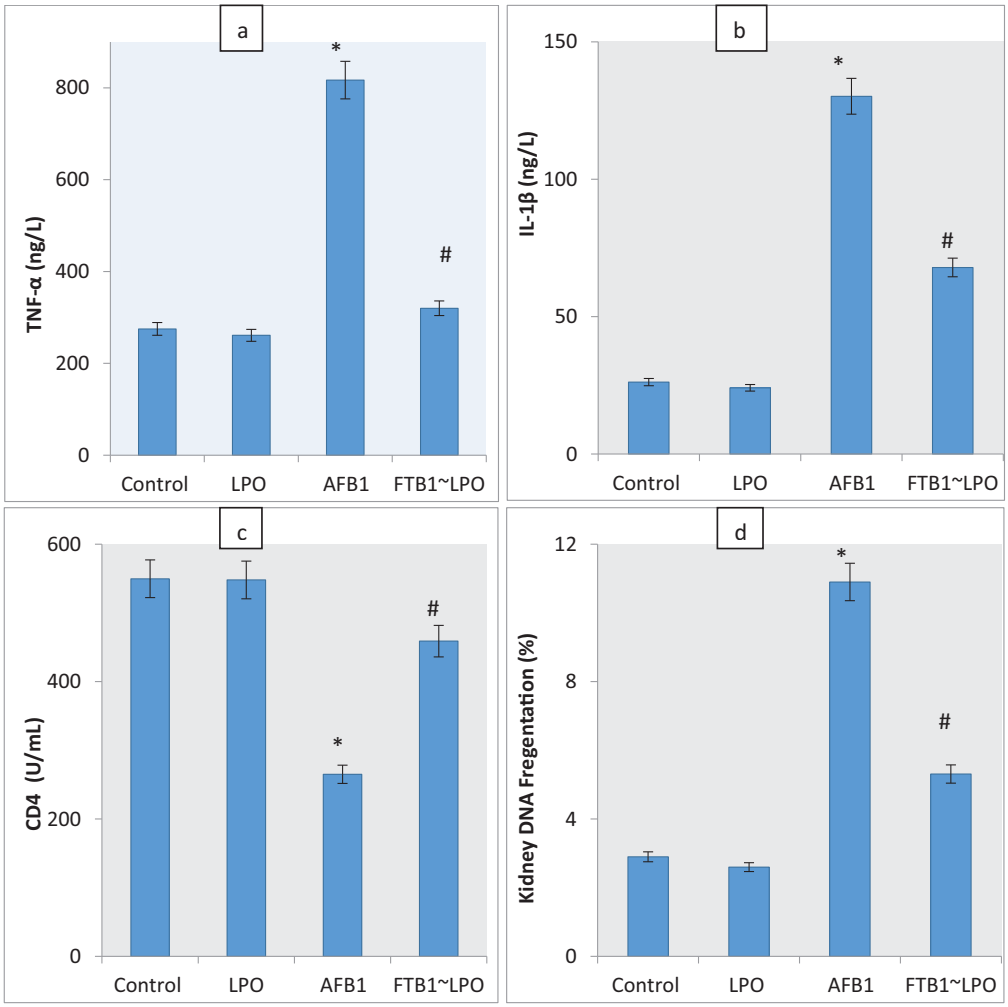
According to Steel and Torrie [21], multiple comparisons between means were performed using one-way analysis of variance (ANOVA) and post hoc (Tukey) at $p \geq 0.05$. The statistical analysis system (SAS) computer software was used for this; copyright (c) 1998 by SAS Institute Inc., Cary, North Carolina, USA.

Results

In comparison to the control group, the obtained results showed a significant increase in TNF-α, IL1β, and DNA fragmentation levels coupled with a significant decrease in CD4-induced AFB1-intoxication. Interestingly, administration of rats with LPO post AFB1 intoxication led to a marked reduction in the measured inflammatory cytokines (TNF-α, IL1β) and kidney DNA fragmentation associated with a significant increase in serum CD4 level to values close to those of the normal control group when compared with AFB1-intoxicated animals (Fig. 1a-d).

The data in Table 1 show that administration of rats with LPO alone did not disturb the serum creatinine,

Figure 1



(a–d) Serum TNF-α, IL1β and CD4 as well as kidney DNA damage of control; AFB1-intoxicated and LPO-treated male albino rats. * is significantly different from the control group, while # is significantly different from the AFB1-intoxicated group ($P \leq 0.05$). AFB1 (aflatoxin B1) and LPO (lactoperoxidase).

urea, urea nitrogen, albumin, uric acid, sodium, potassium, calcium total, magnesium, and phosphorus, while AFB1-treated significantly increased the level of creatinine, urea, urea nitrogen, uric acid, potassium, magnesium, and phosphorus matched with significant degrees of calcium total, albumin, and sodium if contrasted with the corresponding values of the control group. Favorably, treatment of LPO with the AFB1-intoxicated significantly ameliorated the AFB1-induced deteriorations in the mentioned parameters.

Table 2 shows that the intoxication of rats with AFB1 led to a significant elevation in the levels of kidney MDA and NO matched with a marked drop in GSH, SOD, and CAT values compared with that of the control group. Promisingly, animals treated with LPO after receiving an AFB1 had kidney MDA and NO levels significantly lower than those treated with AFTB1, while their GSH, SOD, and CAT values were significantly improved.

Histopathological study

When the control group's renal tissues were examined, they revealed a normal renal cortex with renal corpuscles and proximal convoluted tubules (PCT). The glomerulus, which is formed of a tuft of lobulated capillaries and is encased in Bowman's capsule, was visible as part of the renal corpuscles. More PCTs were observed, each with a small lumen and a lining of pyramidal cells with indistinct cell boundaries. Their cytoplasm was strongly acidophilic with rounded vesicular nuclei (Fig. 2a). The LPO-treated group displayed normal proximal and distal convoluted tubules (DCT) histologically. Some renal tubules were obliterated with congested glomeruli (Fig. 2b). In several regions, the AFB1-treated group displayed a lack of tubular architecture. Some tubules were recognized as basophilic aggregates with poorly defined nuclei, while others were seen as a few dilated tubules with a flattened epithelium. Parts of some tubules' epithelium were sloughing off inside their lumina. Some tubular cells had acidophilic cytoplasm and karyolitic nuclei (Fig. 2c). With a fine nuclear appearance and closely packed renal tubules, the combined AFB1- and LPO-treated group demonstrated considerable improvement in comparison to the AFB1-treated group. However, cytoplasmic vacuolization was observed in a few tubular cells (Fig. 2d).

Discussion

Due to its hepatotoxic, immunotoxic, carcinogenic, and teratogenic effects, AFB1 is the most toxic secondary metabolite generated by fungus, posing serious health concerns to both humans and animals [22–24]. In numerous animal models, the contribution of oxidative stress to AFB1-mediated organ damage has been studied [11,25,26]. Since the liver is the

Table 1 Markers of kidney function of control, aflatoxin B1-intoxicated, and LPO-treated male albino rats

	Control	LPO	AFB1	AFB1∼LPO
Creatinine (mg/dl)	0.49±0.03	0.48±0.06	1.76±0.7*	1.12±0.04#
Blood urea (mg/dl)	32.4±2.3	31.7±1.2	74.2±1.6*	40.2±1.7#
Urea nitrogen (mg/dl)	16.1±1.1	16.0±0.63	37.1±0.85*	19.9±0.93#
Sodium (mmol /l)	133.6±1.4	132.8±1.13	122.6±1.7*	131.1±2.26#
Potassium (mmol /l)	4.5±0.26	4.3±0.18	5.3±0.25*	4.8±0.37#
Magnesium (mg/dl)	2.5±0.05	2.4±0.16	3.2±0.17*	2.6±0.07#
Calcium total (mg/dl)	10.3±0.16	10.2±0.01	7.56±0.27*	9.3±0.3#
Phosphorus (mg/dl)	7.2±0.5	7.1±0.38	9.75±0.25*	8.3±0.35#
Albumin (g/dl)	4.6±0.16	4.7±0.23	3.6±0.13*	4.1±0.15#

Data are presented as mean ±SEM. Data were subjected to one-way ANOVA followed by post hoc (Tukey) test at $P \leq 0.05$. * is significantly different from the control group, while # is significantly different from AFB1 (aflatoxin B1) and LPO (lactoperoxidase).

Table 2 Kidney values of malondialdehyde (MDA), nitric oxide (NO), reduced glutathione (GSH), superoxide dismutase (SOD) and catalase (CAT) of control, aflatoxin B1-intoxicated and LPO-treated male albino rats

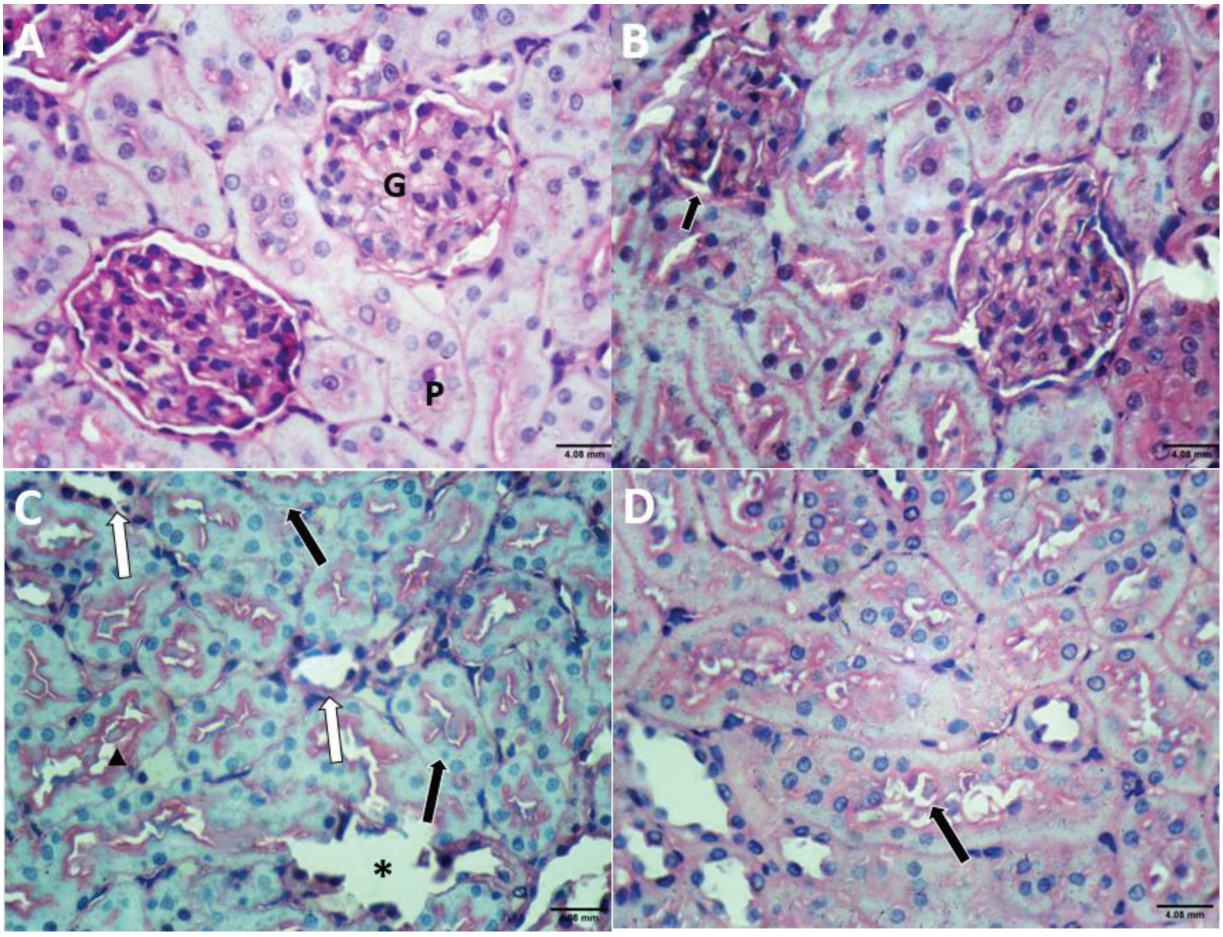
	Control	LPO	AFB1	AFB1∼LPO
MDA (μmol/g tissue)	33.6±0.26	31.2±0.84	64.9±1.4*	39.7±3.2#
NO (μmol/g tissue)	141±10	140±1.7	341±30*	227±7.6#
GSH (nmol/g tissue)	318±27	329±37	215±54*	313±99#
SOD (U/g tissue)	11672±466	13240±646	7346±83*	14093±627#
CAT (U/g tissue)	39.9±2.5	40.1±3.1	10.3±4*	24.1±7.7#

Data are presented as mean±SEM. Data were subjected to one-way ANOVA followed by post hoc (Tukey) test at $P \leq 0.05$. * is significantly different from the control group, while # is significantly different from AFB1 (aflatoxin B1) and LPO (lactoperoxidase).

primary organ for AFB1 bioactivation, the liver has received a lot of attention in these investigations. However, compared with the liver, the kidney has received very little research as an AFB1-susceptible target. In addition, research using animal models in an experimental setting strongly suggested that organ disorders brought on by AFB1 are influenced by oxidative stress [27]. The kidney is a crucial organ that performs numerous crucial tasks, such as maintaining homeostasis and managing the extracellular environment, which includes detoxifying and eliminating harmful drugs and metabolite excretions [28]. Therefore, the current work's goal was to investigate any potential modifying impacts of LPO against oxidative stress and kidney toxicity induced by AFB1 in male rats.

Our results showed that AFB1 produced significantly increased serum creatinine, urea, urea nitrogen, potassium, magnesium, phosphorus, and uric acid levels matched with significantly decreased levels of serum calcium, albumin, and sodium. Increased levels of urea and creatinine indicate a decreased glomerular filtration rate [29]. In addition, our results also showed that AFB1 caused a significant decrease in antioxidant enzymes' activity (CAT and SOD), and significant depletion of GSH, matched with increased MDA production in the renal tissue. These findings agree with those of Sari *et al.* [30] and Wang *et al.* [31]. Based on the results of the serological study demonstrating the existence of greater levels of renal function such as creatinine, urea, urea nitrogen, potassium, magnesium, phosphorus, and uric acid levels. AFB1 treated for an extended period of time causes kidney damage that may include inflammation, cell necrosis, and toxicosis [25,32]. Similarly, increased blood creatinine, urea, urea nitrogen, potassium, magnesium, phosphorus, and uric acid levels matched with significantly

Figure 2



(a–d) Photomicrograph of kidney section of adult male rats: (A) The control group showing normal renal cortical structure with glomeruli (G) surrounded by Bowman's capsule with normal pyramidal cells of PCTs (P). (B) The LPO-treated group showed normal renal tubules with congested glomeruli (black arrow). (C) The AFB1-treated group showed dilated tubules with a flattened epithelium (white arrow); some tubules showed sloughing of parts of their epithelium inside their lumina (arrowhead), and some tubular cells had acidophilic cytoplasm with karyolitic nuclei (black arrow). (D) AFB1∼ LPO-treated group improved the histological pattern of the renal cortex; however, cytoplasmic vacuolization was seen in some tubular cells (black arrow) (H&E 40 x).

decreased levels of serum calcium, albumin, and sodium. According to previous reports, broilers exposed to AFB1 poisoning show signs of decreased renal function [33]. In our study, a significant rise in serum creatinine, urea, urea nitrogen, potassium, magnesium, phosphorus, and uric acid concentrations were also detected following exposure to AFB1. Also, in renal pathological sections and ultrastructural images, corresponding lesions were discovered. Assessments revealed glomerular atrophy, swelling, and hyperplasia of the Bowman's capsule wall in the AFB1-treated group as well as nuclear pyknosis and cytoplasmic vacuolization brought on by AFB1. These findings suggest that AFB1 can cause nephrotoxicity.

When an organism is subjected to detrimental stimuli from the outside, it might experience oxidative stress, which causes the body to produce more ROS such as H_2O_2, superoxide anion (O2•-), and hydroxyl radicals (OH) [34,35]. There have been reports that AFB1 increases ROS generation [24]. Studies have shown that a high ROS, which produces large levels of MDA and causes tissue injury, can damage biological macromolecules like lipids, proteins, and nucleic acids [36]. As a result, the biomarker lipid peroxide MDA is crucial for evaluating oxidative damage. The natural antioxidant defense system's GSH, CAT, and SOD might remove different ROS from the body and guard cells from oxidative harm [37]. Superoxide radicals can be converted by SOD into H_2O_2, which is then processed by CAT and GSH into H_2O and O_2 [38]. In this investigation, AFB1 exposure increased MDA and NO levels in the kidney and markedly decreased CAT, GSH, and SOD activity. Evidently, oxidative stress was generated by AFB1 in rat kidneys. Here, we find that LPO dramatically reduced these nephrotoxic indicators, demonstrating its nephroprotective effect against AFB1-induced renal injury. Numerous studies have demonstrated the nephroprotective properties of LPO in the clinical environment against microalbuminuria in diabetic nephropathy and in vivo rodent models of toxicant-induced renal injury [39] and diabetic nephropathy [40].

In the current investigation, we found that in rats, levels of the proinflammatory cytokines TNF-α and IL1β were significantly increased after AFB1. These results echo those of earlier research that has been conducted [41,42]. Inflammation and oxidative stress are tightly associated with mycotoxin exposure [43]. TNF-α and IL-1β production as well as tissue damage brought on by inflammation have both been linked to AFB1 exposure [44]. In our investigation, rat kidney tissue exhibits upregulated TNF-α expression along with upregulated IL-1β, TNF-α, and HSP70 gene expression levels. TNF-α, which regulates the metabolic responses of another tissue and promotes the synthesis of various cytokines throughout the inflammation process, is the first and most important inflammatory mediator [45]. It was evident from the changes in the levels of inflammatory cytokine expression in the rat kidneys that were exposed to AFB1 and had an inflammatory impact. With the treatment with LPO-treated rat-induced nephrotoxicity by AFB1, TNF-α and IL-1β levels were drastically lowered, indicating that LPO may have anti-inflammatory properties. These findings support LPO's earlier research on its ability to reduce inflammation in rheumatoid arthritis animal models [46], inflammatory bowel disease [47], steatohepatitis [48], alcohol-induced hepatic injury [49], and wound healing [50]. A possible mechanism that could account for LPO's inhibition of the leukocyte infiltration marker MPO is the reported reduction in TNF-α level, which is known to cause leukocyte recruitment by boosting the production of ICAM and P-selectin adhesion molecules [51].

After AFB1 treatment, histological changes in the rat kidney showed acute tubular necrosis, confirming irreversible kidney injury. AFB1 intoxication also demonstrated extreme atrophy of the glomerulus, which was apparent due to its reduced size. Dilated tubules with a flattened epithelium, some tubules showed sloughing of parts of their epithelium inside their lumina, and some tubular cells had acidophilic cytoplasm with karyolitic nuclei. Another sign of AFB1-induced renal necrosis is increased tissue in the interstitium and cellular debris in the tubular lumen. The current study's alterations correspond to those in the report by Wang *et al.* [31].

Conclusion

This study demonstrated that LPO exhibited a therapeutic effect on AFB1-induced nephrotoxicity. This effect was mechanized through the upregulation of renal antioxidant activity, which in turn reduced the oxidative stress deteriorating effects besides its immunoregulatory effect. LPO probably possesses a promising potential for the management of mycotoxin-induced nephrotoxicity.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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