Fucoidan supplementation modulates hepato-renal oxidative stress and DNA damage induced by aflatoxin B1 intoxication in rats

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ABSTRACT

Aflatoxins are a common food contaminant of global concern. Aflatoxin B$_1$ (AFB$_1$) intoxication is associated with serious health hazards. Recently, fucoidan (FUC) has gained much attention from pharmaceutical industry due to its promising therapeutic effects. The impacts of FUC on AFB$_1$-induced liver and kidney injures have not been sufficiently addressed. This research was conducted to evaluate the ameliorative effect of FUC in AFB1-induced hepatorenal toxicity model in rats over 14 days. Five groups were assigned; control, FUC (200 mg/kg/day, orally), AFB$_1$ (50 μg/kg, i.p.), and AFB$_1$ plus a low or high dose of FUC. AFB$_1$ induced marked hepatorenal injury elucidated by substantial alterations in biochemical tests and histological pictures. The oxidative distress instigated by AFB$_1$ enhanced production of malondialdehyde (MDA) and nitric oxide (NO) along with reduction in the reduced-glutathione (GSH), glutathione peroxidase (GSH-Px), superoxide dismutase (SOD), and catalase (CAT) activities. DNA damage in the liver and kidney tissues has been demonstrated by overexpression of proliferating cell nuclear antigen (PCNA). Unambiguously, FUC consumption alleviates the AFB$_1$-induced mitochondrial dysfunction, oxidative harm, and apoptosis. These ameliorated effects are proposed to be attributed to fucoidan's antioxidant and anti-apoptotic activities. Our results recommend FUC supplementation to food because it exerts both preventive and therapeutic effects against AFB$_1$-induced toxicity.

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1. Introduction

Aflatoxins (AFs) are toxic secondary metabolites produced by filamentous fungi, *Aspergillus flavus* and *A. parasiticus* (Huang et al., 2020). AFs are deemed by the Food and Drug Administration (FDA) as inescapable contaminants due to their ubiquitous existence in food, fodders, and feed ingredients that empower hazards on humans' and animals' health (Gell and Carbone, 2019). Among different types of AFs (AFB$_1$, AFB$_2$, AFG$_1$, and AFG$_2$), AFB$_1$ is the most prevailing toxic one (Gell and Carbone, 2019). It has mutagenic (Chen and Liu, 2019), carcinogenic (Taranu et al., 2019), teratogenic (Smith et al., 2017), and an immunosuppressive action (Mohsenzadeh et al.,

2016). Therefore, different organs are affected during AFB$_1$ intoxication. Principally, liver and kidney are the incipient target organs for AFB$_1$ (Karamkhani et al., 2020).

It is well known that AFB$_1$ is biotransformed by liver cytochrome-P450 into AFB1-8, 9- epoxide (AFBO), which is a harmful and extremely active, electrophilic metabolite (Taranu et al., 2019). AFBO is then detoxified by the hepatic glutathione S-transferase system via glutathione conjugation into their non-toxic polar forms that are ready for excretion in bile and urine (Iqbal et al., 2019). Accordingly, most of the substantiation denotes that consumption of intracellular antioxidants systems such as superoxide dismutase (SOD), reduced-glutathione (GSH), glutathione peroxidase (GPx), and catalase (CAT), along with the enhanced generation of free radicals is considered one of the fundamental mechanisms implicated in the AFB$_1$-induced tissue injury (Rotimi et al., 2019). Thereby, the significance of antioxidant supplementations has been highlighted by many studies in order to combat the AFB$_1$-evoked oxidative damage, including that conducted by our group,

which revealed the antioxidant potency of *Spirulina platensis* during AFB_1 intoxication in Nile tilapia (Abdel-Daim et al., 2020c). Curcumin extract has also shown a renoprotective activity in AFB_1-induced oxidative damage in albino rats (El-Mahalaway, 2015).

Fucoidan (FUC) is a water-soluble sulfated polysaccharide extracted from aquatic brown algae; it mainly contains a high proportion of L-fucose-4-sulfate, with smaller amounts of uronic acid, galactose, glucuronic acid, xylose, mannose, rhamnose, glucose, arabinose, and xylose (Wang et al., 2019). Several studies have been published about FUC as a superb natural antioxidant and its potential ROS-scavenging activity (Wang et al., 2016, 2019). There is evidence that the FUC has a protective potential against certain drugs and environmental toxicants-induced hepatorenal injuries such as cyclosporine A (Al-Khatib et al., 2019), carbon tetrachloride (Chale-Dzul et al., 2020), adenine (Wang et al., 2011), N-nitrosodiethylamine (Nakazato et al., 2010), microcystin-LR (AlKahtane et al., 2020), diazinon (Abdel-Daim et al., 2020a), and diabetic hepatorenal injury (Aleissa et al., 2020).

In this context, we hypothesized that FUC supplementation could alleviate the AFB1-induced oxidative damage and boost tissue regeneration through its antioxidant capacity. Therefore, the current investigation commenced assessing fucoidan's modulatory actions on AFB1-triggered oxidative distress and apoptosis in liver and kidney tissues. Liver and kidney biochemical parameters, oxidative state, histological alterations, and proliferating cell nuclear antigen (PCNA) expression were investigated.

2. Materials and methods

2.1. Chemicals

AFB_1 (purity $\geq 99\%$) was obtained from Sigma -Aldrich Chemical Inc. Co. (St. Louis, MO, USA). FUC (*Laminaria japonica*, as 500 mg/capsule) was obtained from Absunutrix Lyfetrition (USA).

2.2. Animal study

From the Egyptian Organization of Biological Products and Vaccines, 40 mature male Wistar Albino rats, weighing 190 ± 10 g were purchased. All animal treatments and experimental procedures were confirmed by the Faculty of Veterinary Medicine's Ethical Committee, University of Suez Canal, Ismailia, Egypt (approval number; 201618). Rats were raised in well-aerated cages one week before commencement of experiment under standard temperature ($25 \pm 2\,°C$), relative humidity of 40–50%, and light (12 h light/dark cycles). A balanced commercial pellet and tap water were offered ad libitum.

After one week of acclimation, rats were randomly assigned to evenly five groups of 8 rats each. The first set received saline as control; the second set (FUC group) was treated with FUC at a dose of 200 mg/kg every day for 14 consecutive days. That regimen was conducted according to Zhang et al. (2005). The third set (AFB_1) was injected AFB_1 at a dose rate of 50 μg/kg, intraperitoneal, twice (on the days 12 and 14) according to our previous study (Abdel-Daim et al., 2020b); the fourth set (AFB_1-FUC100) was given the same regimen of AFB_1 group plus FUC low dose, 100 mg/kg/day, orally for 14 consecutive days; the fifth set (AFB_1-FUC200) was received AFB_1 plus FUC high dose, 200 mg/kg/day, orally for 14 consecutive days.

2.3. Samples collection and processing

The experiment was terminated on the 15th day, and all rats were euthanized under isoflurane anesthesia. Blood sampling was taken straightway from the retro-orbital venous plexus. The sera were separated after centrifugation of blood at $3000 \times g$ for 15 min and then stored at $-20\,°C$ till biochemical assessment. The liver and kidneys were dissected and rinsed off with physiological saline to wash away blood congealment and RBCs for further histological, immunohistochemical, and oxidative biomarkers evaluation.

2.4. Serum biochemical indices

The collected sera were utilized for evaluation of the liver function parameters, including aminotransferase, Aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), and lactate dehydrogenase (LDH) activities. In addition to blood urea nitrogen (BUN) and creatinine analyses to assess the renal function. All procedures were carried out as stated by the manufacturer's (Laboratory Biodiagnostics Co., Giza, Egypt) instructions.

2.5. Oxidative biomarkers valuation

One gram from each tissue specimen was blended in 5 ml ice-cold buffer solution (PBS, 100 mM Na_2HPO_4/NaH_2PO_4; pH 7.4; EDTA, 0.1 mM) and homogenized by a sonicator homogenizer. Then, centrifugation of homogenates at $4\,°C$ ($5000 \times g/30$ min) using a refrigerated centrifuge (Sigma 3-18KS, Sigma, Germany). The supernatant was then harvested and preserved at $-80\,°C$ for the evaluation of MDA, GSH, and NO levels. GPx, CAT, and SOD activities were also determined. All these parameters were estimated by using specific diagnostic kits brought from Laboratory Biodiagnostics Co.

2.6. Histopathological examination

The harvested tissue specimens of the liver and kidney were fixed in formalin 10% for at least 24 h. Subsequently, the specimens were washed out under the overflow of tap water for 10 min then immersed in sequential dilutions of ethanol to be dehydrated. After that, they cleared up in xylene solution before embedding in paraffin. The formed blocks were cut into sections (5 μm thickness) and stained with hematoxylin and eosin (H&E) for inspection under a bright-field microscope.

2.7. Immunohistochemistry study

The liver and kidney paraffin blocks were dewaxed and dehydrated by immersions in sequential ethanol solutions. Following that, antigen retrieval was attained by inundation in the EDTA solution. The endogenous peroxidases were suppressed by using a 3% H_2O_2 solution for 10 min then rinsed three times in PBS 5 min each. Bovine serum albumin (5%) block up solution was then added for 20 min to block nonspecific sites. Incubation with primary antibody anti-PCNA was done (Genemed Biotechnologies Inc., CA, USA, 1:50 dilution) at $37\,°C$ for 2 h. Next, all slides were incubated with avidin-biotin complex (ABC kit, Vector Laboratories) at $37\,°C$ for an hour. Ultimately, the 3,3-diaminobenzidine tetrahydrochloride, DAB (Dako, Japan), and Mayer's hematoxylin were added for visualization of reaction (as brown stain).

2.8. Data statistics

The group sizes were determined from the effect size and variation in the GSH as it is the principal sovereign antioxidant. Sampling at each intervention was based on statistical power of 80%, an expected standard deviation of 15 mg/g tissue, and a desired increment of 35 mg/g tissue. This results in a group size of 7 animals per group. For multiple comparisons between treatment groups, One Way Analysis of Variance (ANOVA) implemented with SPSS (Version 20; SPSS Inc., Chicago, USA) was used. For post hoc comparisons, P-values were adjusted for multiple comparisons according to Tukey. Results are presented as mean and 95% confidence interval (95% CI). The statistical difference was accepted for P-values <0.05. The treatment groups were also compared using principal component analysis (PCA) and multivariate analysis of variance (MANOVA). The R2 value was calculated to give a measure of the goodness-of-fit or amount of variability explained by the model using SAS 9.4 statistical software (SAS Inc., Cary, NC).

3. Results

3.1. Biochemical parameters analyses

As presented in Fig. 1, hepatotoxicity and nephrotoxicity were evoked after AFB_1 exposure evidenced by a dramatic ($P \leq 0.05$) increase in the liver enzymes activities (AST, ALT, ALP, and LDH) and kidney biomarkers levels (BUN and creatinine) in rat serum when in comparison to controls. Conversely, a reduced toxic effect of AFB_1 was observed when AFB_1-treated rats were received FUC (100 or 200 mg/kg), which remarked by amendment of the levels of all measured biochemical parameters. Notably, these data confirm that when FUC extract was administrated at a higher dose, it exerted worthy significant betterment against AFB_1-induced injury than the lower dose. These data suggested

that the improvement of AFB_1-induced injury occurred after FUC administration in a dose-dependent pattern.

3.2. Oxidative damage indices

Lipid peroxidation and oxidative distress indices under the effects of AFB_1 intoxication and/or treatment with FUC in liver and kidney tissues are exhibited in Figs. 2 and 3, respectively. Remarkable increases ($P \leq 0.05$) in MDA and NO levels alongside worthy decreases ($P \leq 0.05$) in the GSH concentration and GPx, SOD, and CAT activities were noticed in liver and kidney tissues in response to AFB_1-intoxication when compared to the control rats. These observations ascertained the existence of oxidative stress. Interestingly, fucoidan's concurrent supplementation could alleviate the $AFB1$-evoked hepatorenal oxidative damage with the more notable ($P \leq 0.05$) improvement of oxidative status in
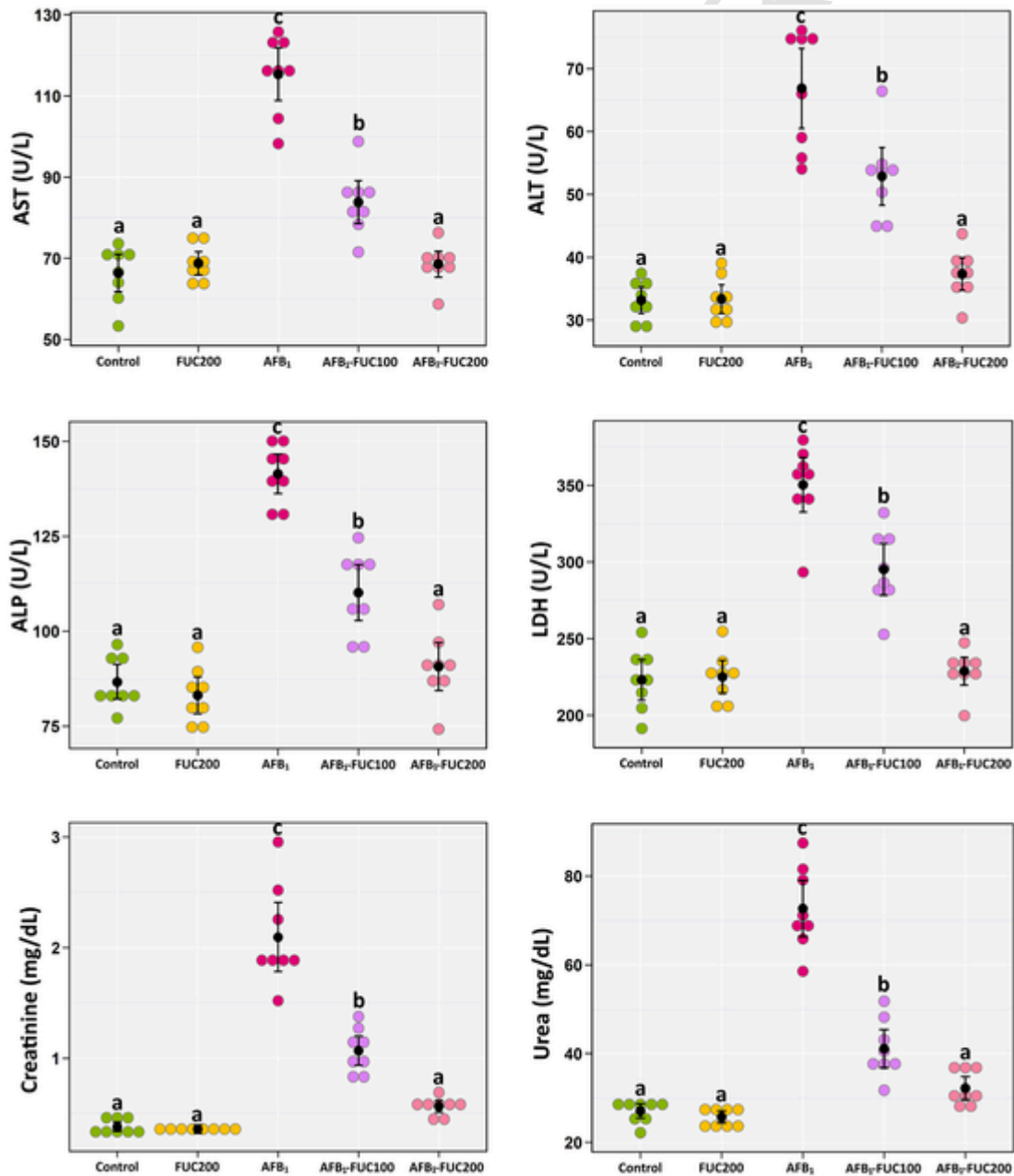

Fig. 1. Dot plots panel with mean (black dot) and 95% confidence interval (the stretching out black lines from the mean) of the liver and kidney metabolites, including aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), lactic acid dehydrogenase (LDH), creatinine and urea in control, fucoidan (FUC200), aflatoxin B_1 (AFB_1), AFB_1-FUC100 combination, and AFB_1-FUC200. Groups with different letters (a–c) are significantly different ($P \leq 0.05$).
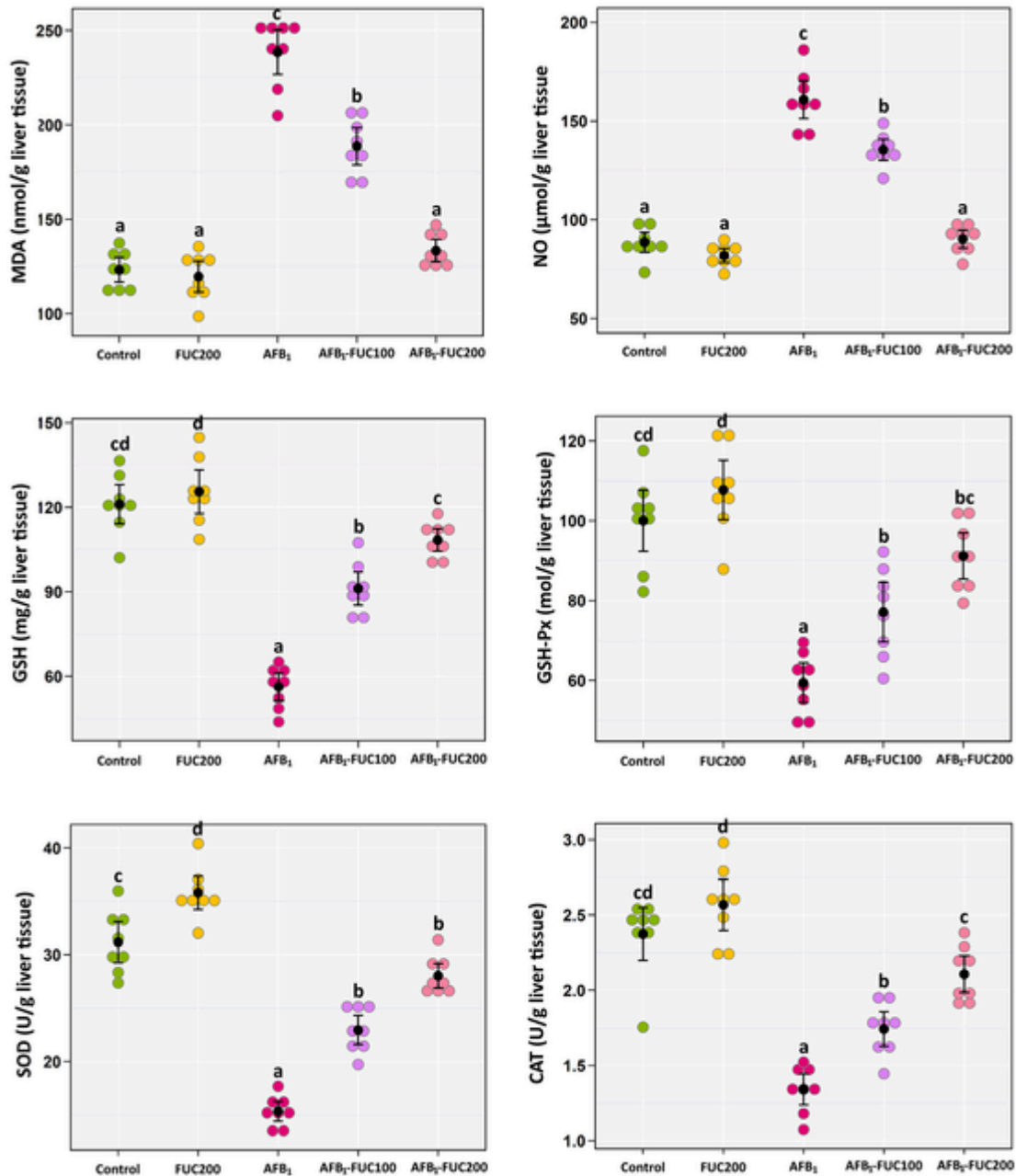
Fig. 2. Dot plots panel with mean (black dot) and 95% confidence interval (the stretching out black lines from the mean) of the liver tissue malondialdehyde (MDA), nitric oxide (NO), reduced glutathione (GSH), glutathione peroxidase (GSH-Px), superoxide dismutase (SOD), and catalase (CAT) in control, fucoidan (FUC200), aflatoxin B_1 (AFB$_1$), AFB$_1$-FUC100 combination, and AFB$_1$-FUC200 combination. Groups with different letters (a–d) are significantly different (P≤0.05).

AFB$_1$-FUC200 group compared with AFB$_1$-FUC100 group. These findings suggested a dose-dependent betterment of FUC contrast to AFB$_1$-prompted oxidative damage.

3.3. Principal component analysis (PCA)

As shown in Fig. 4, PCA results showed that treatment groups were clearly differentiated along the PC1 axis, accounting for the majority (90.0%) of the variation between the treatment groups. In PCA, LDH, and liver and kidney MDA and NO contents seem to drive the AFB$_1$ treated animals to cluster separately from other groups. However, the higher antioxidant activity such as GSH and GSH-Px in AFB$_1$-FUC200 treated animals drive them to aggregate with healthy ones (control group).

3.4. Histoarchitecture pattern of liver and kidney tissues

In order to confirm the above-described findings, the morphological alterations in the liver and kidney tissues after AFB$_1$ and/or FUC treatment were evaluated. Liver sections of control animals had a regular polyhedral normally distributed hepatocytes with normal sinusoidal and central veins pattern, as exhibited in Fig. 5A (Control) and Fig. 5B (FUC 200). Conversely, with respect to AFB$_1$-intoxicated animals (Fig. 5C), the hepatic injury was instigated, which was elucidated by the presence of vacuolar degeneration of hepatocytes with fatty changes. Besides, pyknosis of the nuclei of some hepatocytes and the loss of others. Congestion of central vein with bile duct and Kupffer cell hyperplasia were also observed along with areas of necrosis and fibrosis with enormous inflammatory exudate in the liver's portal areas. Alterna-
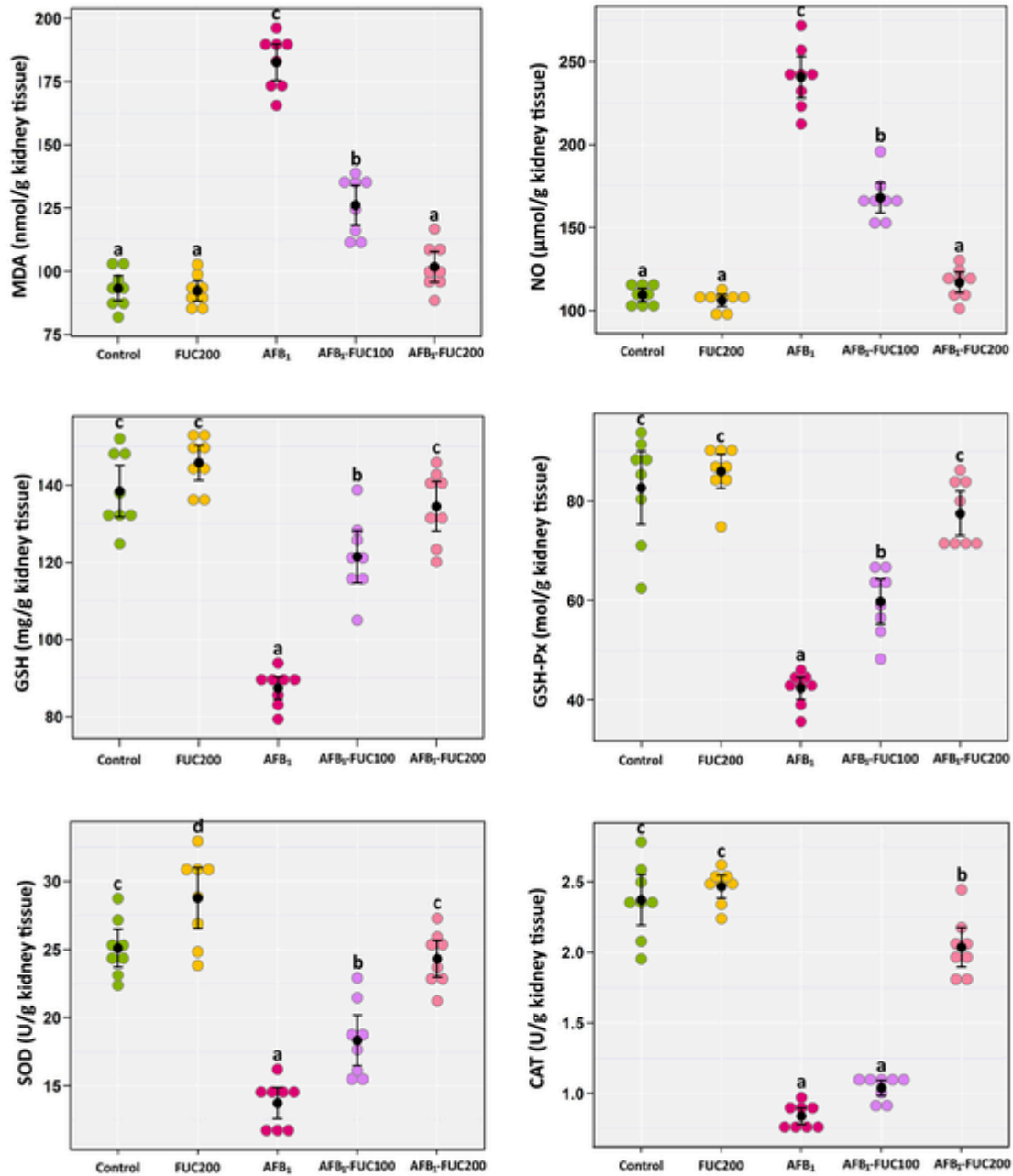
Fig. 3. Dot plots panel with mean (black dot) and 95% confidence interval (the stretching out black lines from the mean) of the kidney tissue malondialdehyde (MDA), nitric oxide (NO), reduced glutathione (GSH), glutathione peroxidase (GSH-Px), superoxide dismutase (SOD), and catalase (CAT) in control, fucoidan (FUC200), aflatoxin B_1 (AFB$_1$), AFB$_1$-FUC100 combination, and AFB$_1$-FUC200 combination. Groups with different letters (a–d) are significantly different (P≤0.05).

tively, in the concurrent sets (AFB$_1$ plus FUC), the portal region showed limited inflammatory cell seepage with un-noticeable centrilobular alterations (Fig. 5D; AFB$_1$-FUC100) and remarkably well-restored hepatic architecture (Fig. 5E; AFB$_1$-FUC200). Our findings expound the ability of FUC to counteract the effect of AFB$_1$ by reducing hepatic histopathological perturbation prompted by this toxin.

In relevance to renal histopathological examination, control and FUC groups (Fig. 6A and B, respectively) showed no alterations; normal architectural pattern of renal corpuscles, tubular brush borders, and interstitium were seen. Contrariwise, the AFB$_1$-intoxicated animals proved to thicken the glomerular basement membrane accompanied by atrophy of glomeruli with obliteration of the capillary lumina and hyaline concernments in the Bowman's capsule. In addition, we observed serious degeneration of the proximal convoluted tubules (PCT) evi-

denced by swelling of lining epithelial cells with cytoplasmic vacuolation and loss of brush border. Other areas displayed necrosis of tubules with cystic dilatation and enhanced cast formation escorted by an inter-tubular hemorrhage and inflammatory cell infiltration (Fig. 6C). Notwithstanding, the synchronous treatment of AFB$_1$ with FUC showed mild degeneration of the PCT with slight inflammatory cell leakage (Fig. 6D; AFB$_1$-FUC100). In comparison, with the remarkable recovery of histological findings, most convoluted tubules were renovated (Fig. 6E; AFB$_1$-FUC200). Accordingly, FUC could extenuate AFB$_1$-induced renal injury in a dose-conditioned pattern. The histological changes clearly mirrored the former biochemical data (Figs. 1–3).
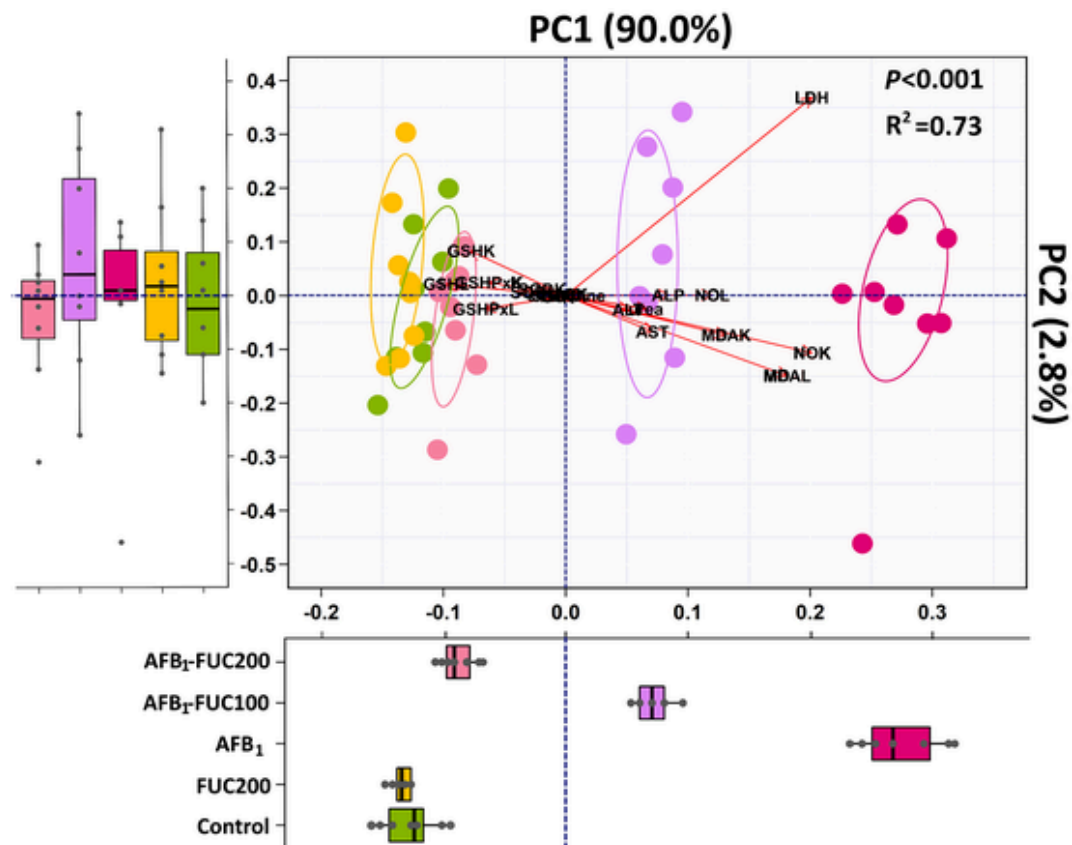
Fig. 4. Principle Component Analysis (PCA) biplot for forty treated rats, control (green dots, n = 8), fucoidan (FUC200; n = 8; yellow dots), aflatoxin B$_1$ (AFB$_1$; n = 8; red dots), AFB$_1$-FUC100 combination (n = 8; purple dots), and AFB$_1$-FUC200 combination (n = 8; pink dots), and their aggregation based on the liver and kidney metabolites (red arrows). Ellipses represent a 95% CI around the cluster centroid. Percentage values indicated on the axes represent the contribution rate of the first (PCA1) and second (PCA2) principal components to the total amount of variation. Box-and-whisker plots shown along each PCA axis indicate the distribution of rats along the given axis. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

3.5. PCNA expression

Expression of PCNA in liver and kidney sections following treatment by AFB$_1$ and/or FUC is displayed in Figs. 7 and 8, respectively. AFB$_1$ obviously up-regulated PCNA expression in the hepatic (Fig. 7C) and renal (Fig. 8C) tissues. Contrary to the AFB$_1$-treated animals, we spotted moderate (Figs. 7 and 8D) and very slight (Figs. 7 and 8E) expressions of the PCNA when rats were conjointly treated by FUC100 and 200, respectively.

From the above data, we confirm the supposition that a high dose of FUC (200 mg/kg) was capable of remarkably preclude the AFB$_1$-mediated DNA damage in hepatic and renal tissues, in a dose-dependent pattern.

4. Discussion

AFs are a group of naturally foodborne toxic metabolites that threaten both humans and animals health (Yilmaz et al., 2018). The most potent toxic aflatoxins are AFB$_1$, produced in *Aspergillus*-contaminated crops under certain temperature and dampness conditions (Li et al., 2018). The AFB$_1$ toxicity is deemed fundamentally in the liver (Xu et al., 2020) and kidney (de León-Martínez et al., 2019) with astounding proofs that lipid peroxidation (LPO), excessive production of oxidants, cellular antioxidant incompetence, and mitochondrial disruption are employed in their pathogenesis (Xu et al., 2020; Yilmaz et al., 2018).

It is well documented that liver is the central organ that incriminated in AFB$_1$ poisoning; where the potent toxic intermediate (exo-8, 9-epoxide; AFBO) is formed (Abdel-Daim et al., 2020b; Marin and

Taranu, 2012) AFBO has the ability to directly fasten the cellular macromolecules including nucleic acids and proteins (Aleissa et al., 2020; Xu et al., 2020) causing excessive generation of ROS such as superoxide anions (O$^-_2$), hydroxyl radicals (OH$^\bullet$), hydrogen peroxide (H$_2$O$_2$), and reactive nitrogen species (RNS) such as nitric oxide (NO) in the cytosol. As a result of disruption of cellular redox homeostasis and induction of LPO of the cell lipid membranes, mitochondrial membrane perturbation, DNA oxidation, protein cross-link, and eventually cell death are occurred (Abdeen et al., 2019b; Abdel-Daim et al., 2020c; Marin and Taranu, 2012; Waheed et al., 2020; Xu et al., 2020). Interestingly, the GSH is the principal sovereign antioxidant (the first line of defense against ROS) that in charge of detoxification of AFBO (via formation of AFBO-GSH conjugate) and neutralization of the generated ROS (Bbosa et al., 2013). However, the overproduction of ROS suppresses the antioxidant capacity of GSH, and oxidative damage is initiated.

Along with the aforementioned, the current results emphasized that excessed ROS and reduced antioxidant capacity played a role in AFB$_1$ intoxication, which elucidated by marked elevations of MDA and NO levels and decreased GSH concentration and GPx, SOD, and CAT activities (Figs. 2 and 3). It has been reported that elevated levels of NO disrupt the mitochondrial electron transport chain (ETC) leading to increased e$^-$ seepage; herein, large amounts of O$^-_2$ are formed (Abdel-daim and Abdeen, 2018; Ge et al., 2017). Additionally, O$^-_2$ and NO may interact together give rise to peroxynitrite ion (ONOO$^-$) that has more deleterious effects on the subcellular molecules (Aleissa et al., 2020). Intriguingly, SOD is an endogenous antioxidant enzyme that represents the first line of enzymatic antioxidant defense required for dismutation of O$_2^{\bullet-}$ to O$_2$ and H$_2$O$_2$ (Abdeen et al., 2019c). The
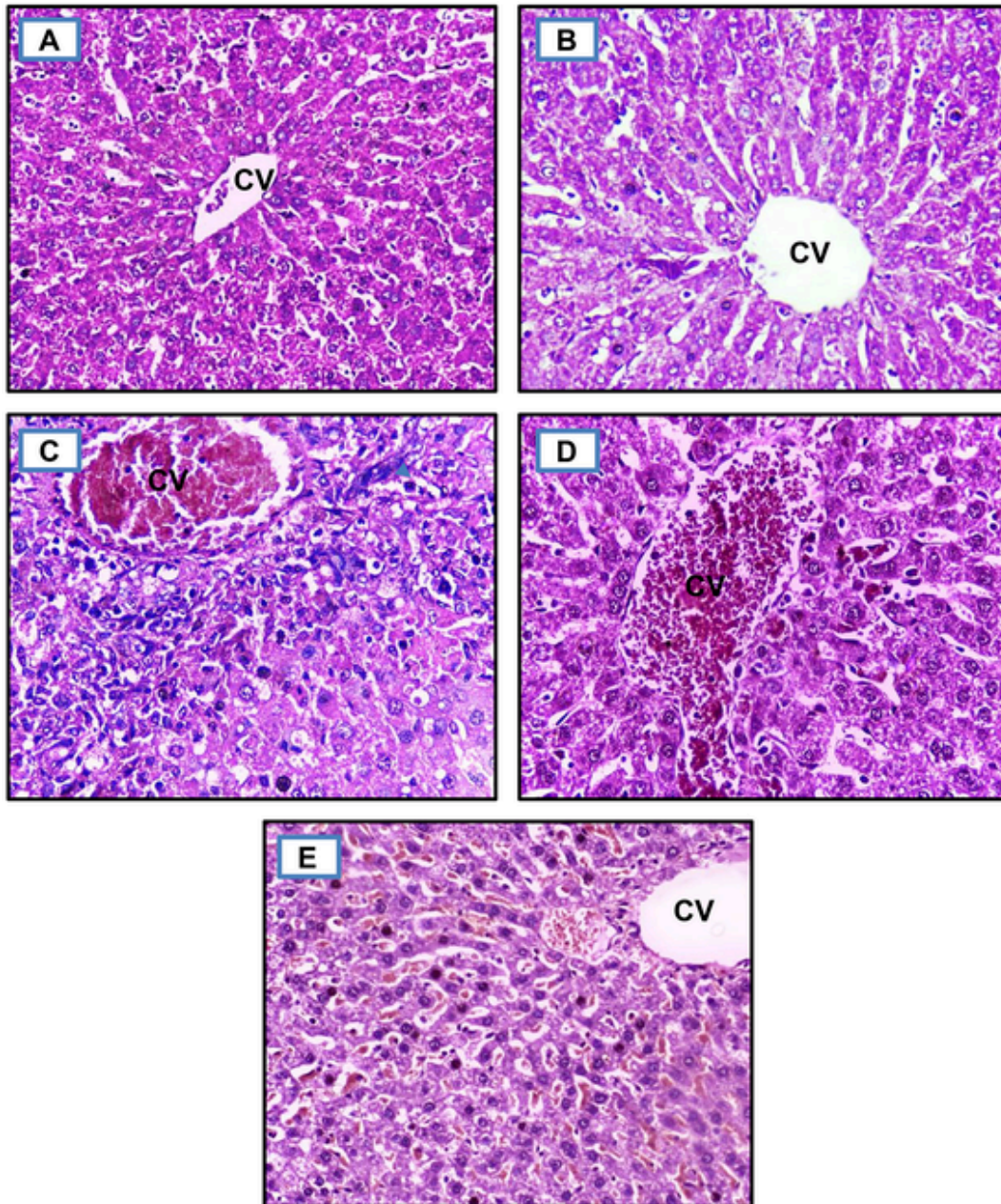
Fig. 5. The effects of the dose-conditioned pattern of FUC co-treatment with AFB_1 intoxication on rat liver. A: Control and B: FUC200 groups exhibit normal liver histological pattern. C: AFB_1-intoxicated rat exhibits, fatty degeneration of hepatocytes with pyknotic nuclei, congestion of central vein, and inflammatory infiltrations. D: AFB_1-FUC100 group, the portal region showed limited inflammatory infiltrations with un-noticeable centrilobular alterations. E: AFB_1-FUC200 group exhibits remarkably well-restored hepatic architecture (CV, central vein; H&E ×400).

generated H_2O_2 was then decomposed by the act of CAT and GPX enzymes into H_2O and O_2. Alternatively, when these enzymes were depleted as reported in AFB_1-exposed group, abundant quantities of OH• were generated by Fenton's reaction. OH• is the central modulator of LPO of the cell membrane (Abdel-Wahhab et al., 2007), which was detected by increased levels of MDA in liver and kidney tissues of the current study. Even worse, MDA itself can further destruct the mitochondrial membrane, cellular proteins, and DNA as well as it can easily passes through the cell membrane and reacts with intracellular biomolecules causing a diffused cellular damage (Abdeen et al., 2020a).

Expectedly, the increased LPO and loss of hepatocyte membrane integrity revealed after AFB_1 exposure might contribute to the unleashing of hepatic enzymes (AST, ALT, and ALP) in the bloodstream raising their serum levels (Aleissa et al., 2020). These findings confirm our previous reports that attested to a positive correlation between MDA level and the increased serum levels of hepatic enzymes (Abdeen et al., 2020a, 2019c, 2019b; Abdel-Daim et al., 2020c, 2019). Eraslan et al. (2017) has also demonstrated significant hepatocellular degeneration with promoted activities of liver enzymes that prove substantial impediment of the liver function during AFB_1-intoxication. Since the ETC was inhibited because of ABF_1-disrupted ROS/antioxidant balance, the aerobic respiration is shifted to an anaerobic pathway leading to an increase in the activity of LDH as recorded in this study. These data are consistent with our previous in vivo study; wherein, we demonstrated increased LDH activity as a result of altered ETC during fipronil insecticide exposure (Abdel-Daim and Abdeen, 2018).

The current study also assured the hurtful effect of AFB_1-induced LPO on the renal epithelial membrane demonstrated by the disintegra-
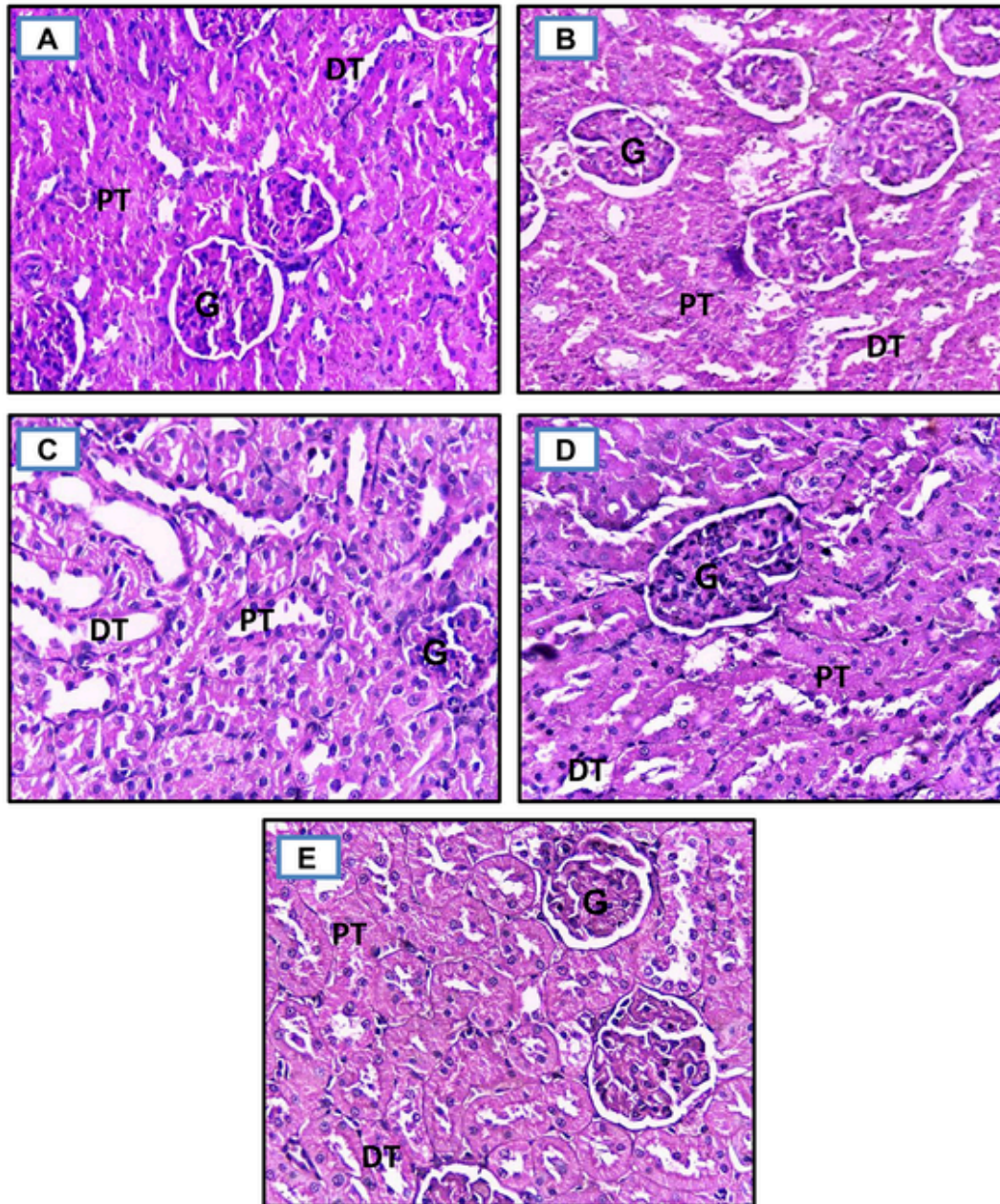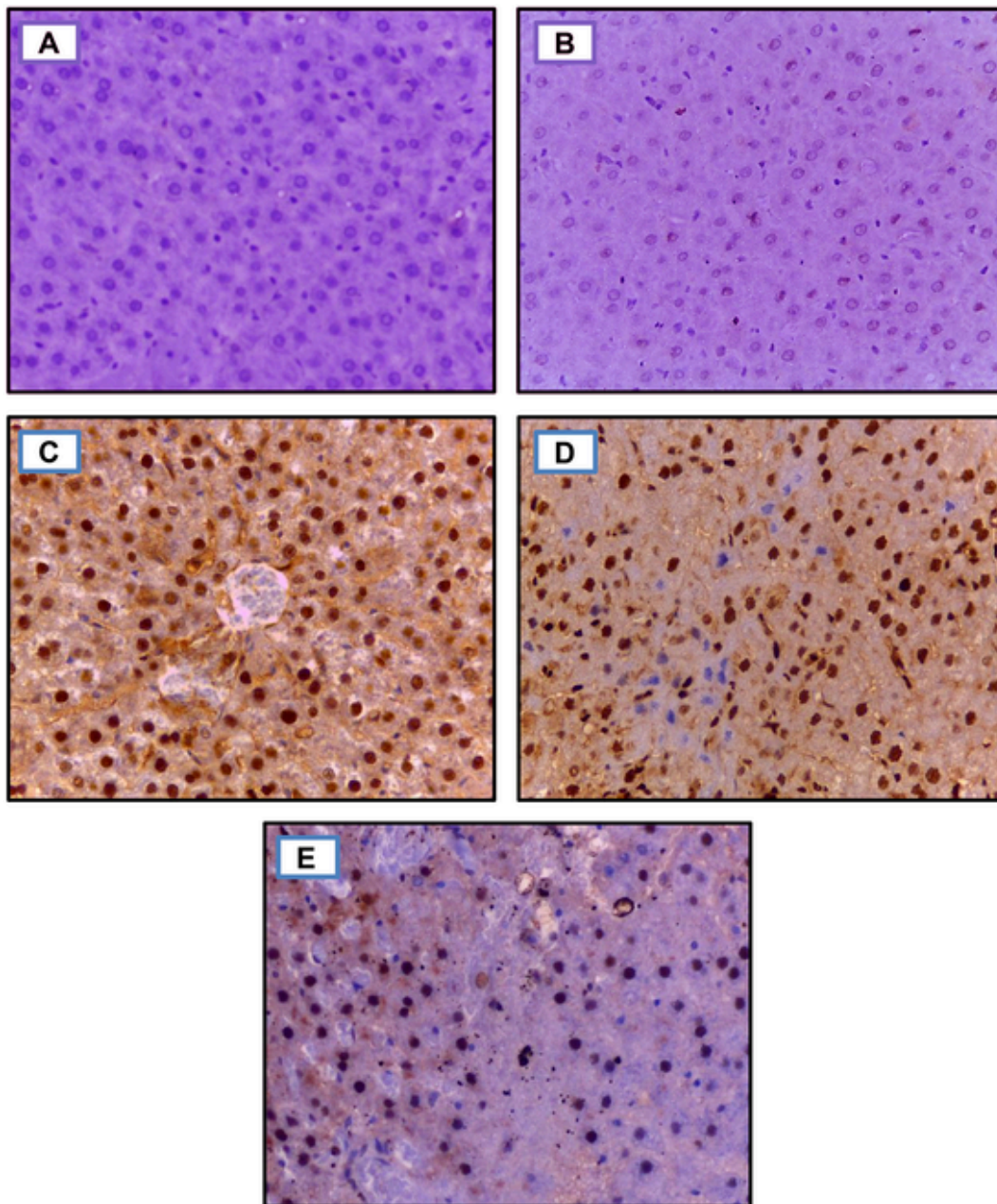
Fig. 6. The effects of the dose-conditioned pattern of FUC co-treatment with AFB_1 intoxication on rat kidney. A: Control and B: FUC200 groups exhibit a normal renal structure. C: AFB_1-treated rat exhibit glomerular atrophy, degeneration of proximal tubules, loss of brush border, tubular dilatation, and cast formation. D: AFB_1-FUC100 group presents mild inflammatory infiltration with mild tubular brush border loss. E: AFB_1-FUC200 shows remarkable recovery of renal histological picture; most convoluted tubules were renovated (G, glomerulus; DT, distal tubules; H&E $\times 400$).

tion of tubular brush border, mainly in the cortical part, as shown in histological inspection (Fig. 8C). Consequently, a tubular impairment occurred indicated by a notable rise of serum urea and creatinine levels (Gowda and Ledoux, 2008). It is well known that mitochondria are the incipient target of oxidative damage. Therefore, due to the high abundance of mitochondria in the proximal tubules, the cortical part of the nephron is the most vulnerable part prone to structural and functional damage by ROS (Huang et al., 2020). Contrariwise, the renal medulla is less susceptible to oxidative damage as their mitochondria can adapt to low cellular oxygen levels and are still able to supply adequate amounts of ATP (Schiffer et al., 2018). Besides, regrettably, kidney PCT relies on the circulating GSH contrasting to other renal parts that can synthesize their own GSH (Ratliff et al., 2016). These data are highly corroborating our previous studies, which indicated the susceptibility of the cortical tubules to the oxidative harm prompted by antibiotics; puromycin (Abdeen et al., 2020b), and gentamicin (Abdeen et al., 2014) and anti-inflammatory drugs; piroxicam (Abdeen et al., 2020a, 2019b, 2019c); and paracetamol (Abdeen et al., 2019a). Taken together, our findings confirmed that the kidney is one of the primary target organs for AFB_1 intoxication and that was in concordance with the data obtained by other investigations (Abdel-Daim et al., 2020d; El-Mahalaway, 2015; Yilmaz et al., 2018). LPO and oxidative damage are also associated with boost production of pro-inflammatory cytokines and severe inflammatory cell recruitment into the liver, and kidney tissues, another implicated mechanism in AFB_1-induced hepatorenal injuries (Aleissa et al., 2020). This finding is in harmony with our histopathological pictures that exhibit inflammatory cell infiltrations.
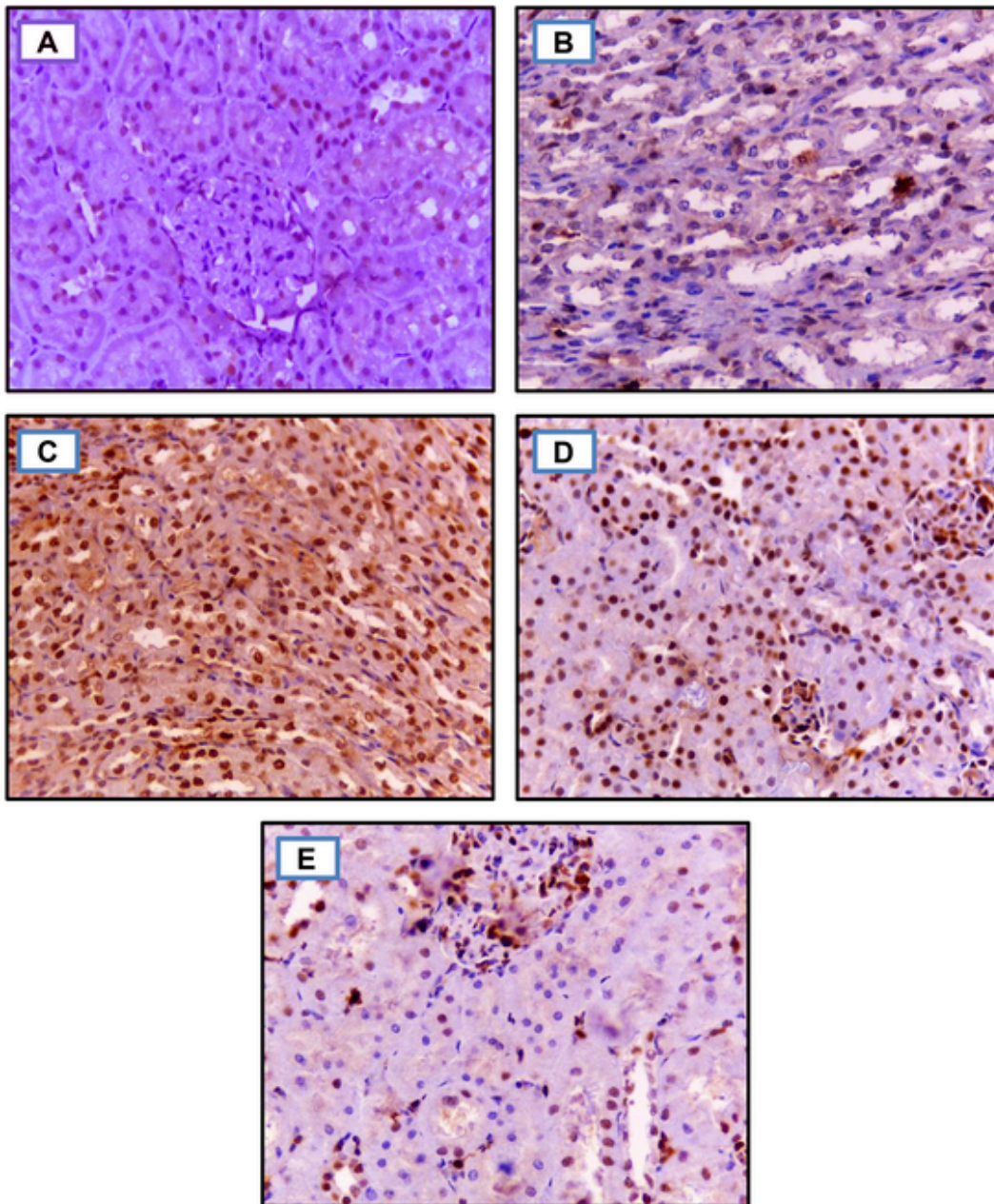
Fig. 7. Effects of FUC dose-conditioned pattern on PCNA expression following AFB$_1$ toxicity on rat liver. A: Control and B: FUC200 groups exhibit passive expression of PCNA. C: AFB$_1$-intoxicated set shows obviously up-regulated PCNA expression. D: AFB$_1$-FUC100, and E: AFB$_1$-FUC200 groups spotted moderate and very slight expressions of the PCNA, respectively. The positive PCNA expression is indicated by a brown coloration of hepatic nuclei. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

It has been reported that AFB$_1$ causes DNA damage through direct binding of AFB$_1$-8,9-epoxide to guanine residue along the DNA double strands forming the predominant DNA adduct, AFB$_1$-N7-guanine (Bedard and Massey, 2006). Accumulative evidence suggests the potential involvement of ROS in DNA oxidation. Therefore, AFB$_1$-enhanced ROS production might be another indirect mechanism by which AFB$_1$ affects DNA integrity (Abdel-Daim et al., 2020d). The present investigation confirms the occurrence of DNA damage in response to AFB$_1$ exposure, which is indicated by up-regulation of PCNA expression, a well-known nuclear biomarker for DNA damage and repair (Strzalka and Ziemienowicz, 2011). Our data have coincided well with those obtained by Li et al. (2019) and Banlunara et al. (2005), who revealed promoted expression of PCNA in hepatocytes challenged with AFB$_1$ exposure.

The physicochemical properties of FUC are the key to their antioxidant activity as molecular weight, polyphenol, and sulfate (−OSO3H) content. Corroborating evidence from several studies strongly indicated that the degree of sulfation immensely affects FUC antioxidant bioactivities (Ma et al., 2017; Rhee and Lee, 2011). FUC active sulfate group (−OSO3H group) had a greater chelating potential against the transition metal ions (such as ferrous or cupper necessary in catalyzing the free radical chain reaction) and form metal complexes. Thereby, the Fenton's reaction is blocked and consequently prevents the generation of OH$^•$ from O$_2$$^{•-}$ and H$_2$O$_2$ (Ma et al., 2017; Wang et al., 2016). Unambiguously, the presence of −OSO3H group allows free confer of electrons and a hydrogen atom (H$^+$) required for free radical stabilization; herein, the oxidative stress is quenched (Ma et al., 2017). Moreover, enormous evidence confirmed fucoidan's capability to up-regulate

Fig. 8. Effects of FUC dose-conditioned pattern on PCNA expression following AFB$_1$ toxicity on rat kidney. A: Control and B: FUC200 groups exhibit passive expression PCNA. C: AFB$_1$-intoxicated set shows obviously PCNA expression. D: AFB$_1$-FUC100, and E: AFB$_1$-FUC200 groups spotted moderate and very slight expressions of the PCNA, respectively. The positive PCNA expression is indicated by a brown coloration of renal nuclei. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

the cellular antioxidant components (Ma et al., 2017; Wang et al., 2010, 2019). By these mechanisms, in the present study, FUC was able to inhibit LPO and decrease NO concentrations along with restoring the GSH, CAT, SOD, and GPx close to their normal values in AFB$_1$-intoxicated animals. That beneficial outcome was demonstrated by a remarkable quell of liver and kidney biomarkers alongside worthy improvements of histopathology picture in a dose-dependent pattern. Our findings agree with the former reports that FUC in a dose-dependent manner could protect against chronic kidney disease in a rat model (Zhang et al., 2003). Another report documented increased antioxidative enzyme activities in catfish fed on FUC supplements (Yang et al., 2014). Remarkably well, FUC also showed a potent protective effect against AFB$_1$-induced DNA damage demonstrated by lowered PCNA expression levels.

In addition to the antioxidant activity, FUC has a renowned potent anti-inflammatory activity through its inhibition of leukocyte recruitment, blocks L-selectin (Fitton, 2011), and disrupt the cell-cell interactions (Corban et al., 2019) indicated by the absence of inflammatory cell infiltration in our histopathology when compared to AFB$_1$-intoxicated animals.

Besides the classical single-variate analysis, we applied supervised PCA for analysis of the effects of AFB$_1$ and FUC interventions on the hepatorenal function and antioxidant system in rats. To the best of our knowledge, this is the first study to use the supervised PCA to evaluate the preventive and therapeutic effects of FUC against AFB$_1$-induced intoxication. Our PCA discriminant analysis depicts the trajectories of each experimental group through space spanned by the biochemical parameters and oxidative damage indices in a less variable-dependent

manner. However, it does not reflect the exact hypothesis testing. The results of PCA showed how the AFB_1 intoxication causes an injurious shift in the hepatorenal parameters and antioxidant system makes these rats differentiated from the healthy rats with roughly 90%. Both single-variate and PCA analysis applied in this study showed marked suppression to the injurious effects of AFB_1 in rats supplemented with FUC through enhancing the antioxidant activity. We found that spatial GSH, NO, MDA, and LDH explained a large proportion of the variance among groups, which is considered a new finding. Interestingly, PCA revealed an increased variability among AFB_1-FUC100 rats and supported the dose-dependent pattern concept. Therefore, the supervised PCA provides an important method to understand the mechanisms of hepatorenal injuries derived by AFB_1 and the protective and therapeutic effects of FUC against AFB_1-induced toxicity.

5. Conclusions

Overall, AFB_1 exerted severe hepatorenal injuries attributed to oxidative stress, lipid peroxidation, and DNA damage, which are considered the main mechanisms implicated in its toxicity. In a dose-conditioned manner, FUC supplementation could abrogate the AFB_1-inflicted oxidative and DNA damage in the liver and kidney tissues. It restores the hepatorenal markers, enhances the antioxidant enzyme activity and the other indices of oxidant status of AFB1-exposed animals. The supervised PCA determined the GSH, NO, MDA antioxidant system, and LDH as the best combination suited to describe the differences in AFB_1-induced toxicity and preventive effect of FUC among our groups. Overall results suggest additional of FUC a food supplement could be an efficient protective and therapeutic strategy against AFB_1 toxicity.

Abbreviations

AFB_1
  aflatoxin B_1
AFBO
  AFB1-8, 9-epoxide
AFs
  aflatoxins
ALP
  alkaline phosphatase
ALT
  alanine aminotransferase
AST
  aspartate aminotransferase
CAT
  catalase
ETC
  electron transport chain
FDA
  Food and Drug Administration
FUC
  fucoidan
GSH
  reduced-glutathione
GSH-Px
  glutathione peroxidase
H_2O_2
  hydrogen peroxide
LDH
  lactate dehydrogenase
LPO
  lipid peroxidation
MDA
  malondialdehyde
MMP
  mitochondrial membrane permeabilization

NO
  nitric oxide
OH
  hydroxyl radicals
PCNA
  proliferating cell nuclear antigen
PCT
  proximal convoluted tubules
ROS
  reactive oxygen species
SOD
  superoxide dismutase

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Josephine et al., 2007
Kang et al., 2008
Kawano et al., 2007
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Ravinayagam et al., 2012
Tedesco et al., 2004
Wang et al., 2014

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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