Ameliorative Effect of Nanocurcumin and/or Saccharomyces Cell Wall Against Aflatoxicosis in Broilers

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Abstract

Background

The adverse effect of aflatoxin in broilers well known. However, dietary supplementation of nanocurcumin and/or *Saccharomyces* cell wall may decrease the bad effect of aflatoxin B1 because of their bio-adsorbing feature of the functional ingredients in Yeast Cell Wall and the detoxification effect of curcumin nanoparticles. The goal of this study was to see how nanocurcumin and/or *Saccharomyces* cell wall supplementation and/or aflatoxin affected broiler development, blood and serum parameters, carcass trait, histology, immune histochemistry, liver gene expression, and aflatoxin residue in the liver and muscle tissue of broilers. Broiler chicks with one day old were distributed into five groups according to nanocurcumin and/or saccharomyces cell wall with aflatoxin supplementation. The G1 group was given a formulated diet without any supplements. The G2 group was supplemented with aflatoxin (0.25 mg/kg diet) in the formulated diet. The G3 group was supplemented with aflatoxin (0.25 mg/kg diet) and *Saccharomyces* cell wall (1Kg/Ton diet) in the formulated diet. The G4 group was supplemented with aflatoxin (0.25 mg/kg diet) and nanocurcumin (400 mg/kg) in the formulated diet. The G5 group was supplemented with aflatoxin (0.25 mg/kg diet) and *Saccharomyces* cell wall (1Kg/Ton diet) with nanocurcumin (200 mg/kg) in the formulated diet.

Results

Aflatoxin supplementation had a detrimental impact on the growth performance, blood and serum parameters, carcass trait, and aflatoxin residue in the liver and muscle tissue of broilers, according to the results of this study. In addition, aflatoxin supplementation led to a liver injury that was indicated by serum biochemistry and pathological lesions in the liver tissue. Moreover, the intestinal villi, length, and width were affected. The expression of some genes in the liver tissue was affected.

Conclusion

Saccharomyces cell wall alone or with nanocurcumin attenuated these negative effects and anomalies, and improving all of the above-mentioned metrics.

Background

Aflatoxins are secondary metabolites of mycotoxins, which are naturally occurring and derived from *Aspergillus flavus* and *Aspergillus parasiticus*. It's known to have very toxic and carcinogenic impacts. Among the different serious aflatoxins (B1, B2, G1, and G2, M1, M2), aflatoxin B1 (AFB1) is the most harmful one. It is also considered a Group I carcinogen. Significant economic losses are posed due to human and animal consumption of the food or feed contaminated by AFB1 as its administration exhibits harmful hepatotoxic, teratogenic, mutagenic, and carcinogenic effects on humans and many species of livestock [1]. The most important economic effect of aflatoxicosis in broilers is the decrease in body weight gain, probably caused by disturbances in protein metabolism [2, 3]. On the other hand, aflatoxin has a bad effect on relative organ

weight [4, 5], especially on the liver tissue [6, 7]. Moreover, aflatoxin affects hematological [8, 9] and biochemical [10] parameters.

Curcumin or diferuloylmethane is a naturally occurring low molecular weight polyphenol, which is considered as the active principle substance of the plant *Curcuma longa* (turmeric's rhizome) [11]. Previous studies have proven that curcumin can prevent hepatotoxic and carcinogenic effects of AFB1 in rats [12]. Curcumin can inhibit liver CYP450 isozymes, so it could be a potentially favorable chemopreventive agent for many carcinogenic and toxic agents like AFB1 [1]. But the low oral bioavailability of curcumin due to low absorption, fast metabolism, and fast systemic elimination from the body has been a major problem [13]. Thus, **Sayrafi et al.** [14] and **Heidary et al.** [15] said that utilizing curcumin nanoparticles improves its bioavailability in the body, increasing solubility in water. Curcumin nanoparticle formulations cause a 10-14-fold higher absorption rate compared with the same oral dose of free curcumin.

On the other side, prebiotics is the most widely used component in the production of functional foods today [16]. Yeast (*Saccharomyces cerevisiae*) cell wall (YCW) as a feed additive containing two main prebiotic components (mannan oligosaccharides and 1,3/1,6 β -glucans) has been used in poultry production, improving performance by increasing average daily gain, improving the feed conversion, and enhancing immunological response [17]. Supplementing diets with yeast cell walls increased weight gains and improved feed conversion [17]. A trial of using a cell wall of yeast to minimize the adverse effects of aflatoxin-contaminated diets in broiler chicks resulted in a significant increase in BW gain and improved feed consumption [18].

This work was carried out to investigate the effect of dietary supplemental nanocurcumin and/or saccharomyces cell wall extract on the growth performance, some carcass traits, hematological parameters, some blood biochemical changes, some gene expressions, and some organs histopathology of broiler chicks fed on aflatoxin containing diet.

Results

Clinical signs and postmortem lesions of the aflatoxicated broilers

After two weeks from the beginning of the trial, clinical symptoms of the aflatoxicated broilers were observed. The observed clinical symptoms are retarded growth, legs paralysis and lameness, wings paralysis, whitish diarrhea, and eye lesions. There is no mortality in the aflatoxicated broilers during this trial. At the end of the trial, the euthanized aflatoxicated broilers showed enlarged liver and kidney with generalized congestion in the muscle tissue. On the other side, the treated aflatoxicated groups (G3, G4, and G5) showed normal appearance of the internal organs and muscles.

Growth performance.

The effect of dietary aflatoxin, nanocurcumin and/or saccharomyces cell wall supplementation on the growth performance of broilers has been shown in Table (1). The mean values of final body weight, BWG, and feed intake in the aflatoxin-fed group (G2) were significantly ($P \leq 0.05$) decreased than that of the control group (G1). Dietary supplementation of nanocurcumin and/or *Saccharomyces* cell wall lowered the negative effect of aflatoxin on the final body weight, BWG, and feed intake of broilers. As shown in the table, the combination

between nanocurcumin and *Saccharomyces* cell wall had the highest effect, except in case of feed intake, both the combination between nanocurcumin and *saccharomyces* cell wall as in (G5) and the supplementation of *Saccharomyces* cell wall only as in (G3) shown the same effect. On the other hand, the mean value of FCR in the aflatoxin-fed group (G2) was significantly ($P \leq 0.05$) increased compared to other groups.

Carcass traits

Effect of dietary aflatoxin, Nano curcumin, and/or *Saccharomyces* cell wall supplementation on some carcass traits of broiler as a percent of live body weight showed in Table (2). There was a significant ($P \leq 0.05$) decrease in the dressing percentage and leg muscle in the G2 when compared with the other groups. Moreover, there was a significant ($P \leq 0.05$) increase in dressing percentage in the G5 when compared with G2 and G3. On the other side, there was an insignificant ($P \leq 0.05$) increase in the dressing percentage in G5 when compared with G1 and G4. Regarding the result of the breast muscle, there was a significant ($P \leq 0.05$) increase in the G5 when compared with G1 and G2 (control negative and control positive groups), but non-significant ($P \leq 0.05$) when compared to G3 and G4. Moreover, the relative weight of the liver in the G2 showed a significant ($P \leq 0.05$) increase when compared with the other groups and there was an insignificant ($P \leq 0.05$) decrease between G1 when compared with G4, G3, and G5. Meanwhile, thymus relative weight showed a significant ($P \leq 0.05$) increase in the G3 when compared with the G1 but was non-significant ($P \leq 0.05$) when compared with the other group. Regarding the result of relative organ weight of spleen, there was a significant ($P \leq 0.05$) decrease and insignificantly increase in the G1 compared to G2 and other remaining groups, respectively. On the other hand, the relative weight of proventriculus and bursa was insignificantly ($P \leq 0.05$) increased in G2 when compared with the other group, while there was an insignificant ($P \leq 0.05$) increase in the relative weight of gizzard in G5 compared with the other groups.

Hematological parameters

Effect of dietary aflatoxin, Nano curcumin, and/or *Saccharomyces* cell wall supplementation on hematological parameters of broiler shown in Table (3). There was a significant ($P \leq 0.05$) decrease in RBCs count, HCT, WBCs count, and LYM in the G2 when compared with the other groups. On the other side, there was an insignificant ($P \leq 0.05$) decrease in Hb concentration in the G2 compared with the other groups. Regarding the result of HCT (%), there was an insignificant ($P \leq 0.05$) decrease and increase in the HCT (%) in G3 when compared with G1, G5, and G4, respectively. Meanwhile, HET, ESI, and MON count show a significant ($P \leq 0.05$) increase in G2 compared with the other groups.

Biochemical parameters

Effect of dietary aflatoxin, Nano curcumin, and/or *Saccharomyces* cell wall supplementation on some biochemical parameters of broiler shown in Table (4). There was a significant ($P \leq 0.05$) increase in the ALT, AST, and ALP activities in the G2 (control positive) when compared with the other groups. On the other hand, there was a significant ($P \leq 0.05$) decrease in the ALT activity in the G4 and G5 when compared with the G1 group (control negative). Meanwhile, AST and ALP activities were significantly ($P \leq 0.05$) increased in the G4 and G5 when compared with the G1 group. Regarding results of total protein, albumin, and globulin, there were significant ($P \leq 0.05$) decreases in these all parameters in the G2 when compared with the G1. On the other side, there were significant and insignificant ($P \leq 0.05$) increases in the total protein and globulin and albumin,

respectively, in the G4 and G5 when compared with the G1. Regarding results of the kidney function, the concentrations of the urea and uric acids were insignificantly ($P \leq 0.05$) increased in the G2 when compared with the other groups. On the other side, the creatinine concentration was significantly ($P \leq 0.05$) increased in the G2 when compared with G1 but non-significant ($P \leq 0.05$) when compared with the other groups.

Histopathology of the liver and intestine:

Histopathology of the hepatic tissues of different treated groups was illustrated in Figures (1), which fed on the aflatoxin-free diet, aflatoxin, nanocurcumin and/or *Saccharomyces* cell wall. It was observed that the broiler chicks group was fed an aflatoxin-free diet showing normal histology in which the normal hepatocytes (H) and around the portal area (PA) consisted of the bile duct, portal vein, hepatic arteriole branch (Figure 1A). Conversely, the addition of aflatoxin had an adverse effect on liver hepatobiliary structures tissues histopathology where photomicrograph of liver of aflatoxin group showed marked periportal hepatocytic cells necrosis was observed in addition to mononuclear cells infiltration, fibrosis of the portal area and regenerative (PA) with newly formed bile ducts and mononuclear cells infiltration within the portal area beside wide periportal hepatocytic cells necrosis (Figure 1B). Meanwhile, the photomicrograph of livers of broiler chicks fed aflatoxin with *Saccharomyces* cell wall showing showed a decrease in the periportal hepatic necrosis normal hepatocytes (H) and portal area (PA) with slight infiltration of ed with mononuclear cells (Figure 1C). On the other side, the photomicrograph of livers of aflatoxin+ Nano group showing showed the hepatocytes (H) with multifocal mild cytoplasmic sharp outlines vacuoles referring to fatty change and normal portal area. (PA) (Figure 1D). Meanwhile, photomicrograph of liver of aflatoxin+ and combination group showing normal histology including the hepatocytes (H) and portal area (PA), which consisting of the bile duct, portal vein, hepatic arteriole brunch (Figure 1E). Quantities scoring of the hepatic lesions showed a marked increase in the lesions score within G2, and decreased in G3 and G4, with a marked decrease in G5 (Figure 1F).

On the other side, it was observed that the photomicrograph of the intestine (jejunum jejunal sections) of the control group showing showed normal histology of intestinal villi with normal pseudostratified epithelium with goblet cells (Fig 2A). Conversely, the addition of aflatoxin had an effect on intestinal tissues histopathology where photomicrograph of the intestine (jejunum section) of aflatoxin group showing showed mucosal necrosis of the intestinal villi with the presence of necrotic core associated with blunting and with villous atrophy and focal basophilic regenerated epithelium (Fig 2B). Meanwhile, the photomicrograph of the intestine (jejunum section) of the aflatoxin + *Saccharomyces* group showing showed a marked decrease in mucosal atrophy with an increase in villi length (Fig 2C). On the other side, the photomicrograph of the intestine (jejunum section) of the aflatoxin + Nano group showing showed a mild degree of degenerative and desquamative changes within the intestinal villi with an improvement of villi length. (Fig 2D). While photomicrograph of the intestine (jejunum section) of aflatoxin + combination group showing showed normal histology of intestinal villi with marked improvement of the thickness length of intestinal villi and structure of the lining mucosa (Figure 2E).

Intestinal morphometrical data of intestinal sections of different treated groups showed marked shortening of villi length in aflatoxicated birds with decrease both crypt depth of ratio between crypt depth and villi length ratio when compared with their corresponding data of normal birds. Treatment of aflatoxicated birds with either *saccharomyces* or nanocurcumin or *Saccharomyces* separately showed an increase of intestinal length

and crypt depth in comparison with diseased birds. Interestingly, treatment of diseased birds with a combination of nanocurcumin and *Saccharomyces* demonstrated marked retrieval of intestinal parameters including villi length, crypt depth, and their ratio to the normal limits (Table 5).

Immunohistochemical study:

Immunohistochemical study of the broilers livers was showed in Figure (3), which fed on aflatoxin free diet, aflatoxin, nanocurcumin and/or *Saccharomyces* cell wall.

It was observed that the broiler chicks group was fed an aflatoxin-free diet showing mild immunostaining of NFkB P65 antibody within the hepatocytes (Figure 3A). Conversely, the addition of aflatoxin sharply upregulated the NFkB P65 in the liver tissue where (Figure 3B) showed marked cytoplasmic and nuclear expression of NFkB P65 antibody within the hepatocytes. Meanwhile, the expression of the NFkB P65 in the liver tissue of broiler chicks fed aflatoxin either with *Saccharomyces* cell wall or with nano showing decrease the expression of NFkB P65 antibody within the hepatocytes (Figure 3C and 3D). On the other side, the expression of the NFkB P65 in the liver tissue of the aflatoxin+ combination group showed a marked decrease for the upregulation of the NFkB P65 antibody within the hepatocytes (Figure 3E). Percentage of NFkB P65 positive cells was markedly increase in G2 and decreased in G3, G4, and G5 (Figure 3F).

Expression of *CYP1A1* and *Nrf2* G5nes within the liver tissue of broilers:

The response of some G5nes related to carcinogen and antioxidant as *CYP1A1* (Cytochrome P450 Family 1 Subfamily A Member 1) and *Nrf2* (Nuclear factor erythroid 2-related factor 2), respectively, were illustrated in Fig. (4). The expression of *CYP1A1* G5ne was markedly increased in the aflatoxin group (G2) in comparison with the control (G1) group ($P > 0.005$). Marked decrease of this G5ne with supplementation of *Saccharomyces* cell wall and/or nano relevant with the aflatoxin group ($P > 0.005$). Liver tissues of aflatoxin animals revealed a marked decrease of *Nrf2* than the normal group ($P > 0.005$), which increased to the normal limits of control groups and significantly increased in aflatoxicated-bird supplemented with *Saccharomyces* cell wall and/or nano than aflatoxin group ($P > 0.05$).

Aflatoxin residue within the liver and muscle tissue of broilers:

Aflatoxin residues in the liver and muscle tissue at the end of the experiment in all groups and at two weeks after the end of the experiment in the liver tissue of the G2 (aflatoxin group) were illustrated in Table (6). The aflatoxin residue at the end of the experiment in both liver and muscle tissue was markedly increased in the aflatoxin group (G2) in comparison with the other groups ($P \leq 0.05$). Marked decrease of this residue with supplementation of *Saccharomyces* cell wall relevant with the aflatoxin group ($P \leq 0.005$). Liver tissue of aflatoxin animals at 2 weeks after the end of the experiment revealed a marked decrease of aflatoxin residue than G2 at the end of the experiment ($P \leq 0.05$).

Discussion

Clinical signs and postmortem lesions of the aflatoxicated broilers

The clinical symptoms of the aflatoxicated broilers in the present trial are compatible with the results of **Salim et al. [19]** who reported that the aflatoxicated birds show retarded growth, legs paralysis, and lameness, wings paralysis. These results may be attributed to the effect of aflatoxin on the nervous system as sciatic nerve, leading to nerve injury [20]. On the other side, there is whitish diarrhea that indicates infection because of immune deficiency that leads to pathogenic microbes flushing [21]. **Tessari et al. [20]** reported that aflatoxin decreases protein because of liver injury that leads to immune suppression. Regarding results of mortality and lesion of euthanized birds, there is no mortality in the aflatoxicated broilers with enlarged liver and kidney accompanied with generalized congestion in the muscle tissue. These results were confirmed by the results of **Salem et al. [19]**.

Growth performance

The results obtained regarding the significant reduction in the mean values of final body weight, BWG, and feed intake in the aflatoxicated group (G2) are in harmony with previous studies reporting the negative effects of aflatoxin on the growth performance [5, 18, 22]. These results are incompatible with the results of **dos Santos et al. [23]** who reported that AFB1 has no deleterious effects on the broilers' performance. This difference may be attributed to the period of the aflatoxin exposure (experimental period) where in our study the experimental period was 35 days. On the other side, dietary supplementation of nanocurcumin or *Saccharomyces* cell wall lowered the negative effect of aflatoxin on the growth performance parameters of broilers that was assured by the results of **Gowda et al. [24]** and **Liu et al. [25]**, respectively. In contrast, **Nemati et al. [26]** reported that glucomannan administration does not prevent or decrease the aflatoxin deleterious effects on broilers' growth parameters. Moreover, **Santin et al. [27]** found that *saccharomyces* cell wall does not ameliorate the negative effect of the AFB1 on weight gain but improves FCR of the broilers. Really, to our knowledge, there are no publications on the combination of both nanocurcumin and *Saccharomyces* cell walls on the aflatoxicated birds.

Carcass traits

The result in the present study showed a decrease in the dressing percentage in the aflatoxicated group (G2) comparing with the other groups. These results are in line with the findings of **Pasha et al. [28]** and **dos Santos et al. [23]**. On the other side, the significant effect of *Saccharomyces* cell wall on carcass yield is compatible with the results of **dos Santos et al. [23]** who reported that the *Saccharomyces* cell wall improve carcass yield and characteristics of aflatoxicated broilers. Moreover, the significant increase of the breast muscle in the aflatoxin +combination group (G5) may be attributable to the favorable effect of nanocurcumin in elevating the crude protein content of broiler breast meat [29] and protective effects of the functional ingredients in YCW (beta-glucan and mannan oligosaccharides) [30]. As well, the relative weight of the liver in the aflatoxicated group (G2) shows a significant increase when compared with the other groups. These findings are similar to **Dhanapal et al. [31]** who reported that there was a significant increase in relative liver weight in broilers fed on a basal diet + 1 ppm aflatoxin from day 14 to 28 post-treatment. In the current study, dietary supplementation of *Saccharomyces* cell wall extract significantly increased thymus relative weight as reported by **Mendieta et al. [32]**, but regarding the relative weight of bursa, the findings are in contrast with the present study as bursa is insignificantly increased in the Afla treated group (G2).

Hematological parameters

Hematological toxic effects of AF are a well-investigated and well-known subject. These toxic effects were also clearly observed in the present study. There was a significant decrease in RBCs count, HCT, WBCs count, and LYM in the G2 when compared with the other groups. These results are compatible with **Basmacioglu et al. [33]** who reported the same result when AF was incorporated into the diet of broiler chicks. Aflatoxin treatment was reported to induce a significant increase in HET, ESI, and MON count [8] and this is what our study proves, HET, ESI, and MON count show a significant increase in G2 compared with the other groups. On the other hand, the beneficial effect of yeast cell wall extract on the hematology of broilers fed on AF treated diet was reported in a previous study [32]. Moreover, **Rahmani et al. [34]** reported that nanocurcumin improves hematological parameters of broilers chicks under cold stress. This improvement may be attributed to the antioxidant effect of the nanocurcumin [35].

Biochemical parameters

There was a significant increase in the ALT, AST, and ALP activities in the Afla group as reported by previous studies [1, 19, 36, 37], and this is evidence of the existence of liver injury due to toxicity. Conversely, these outcomes are different from this previous study [38], which explained that AFB1 does not alter serum ALT and AST activities. Similar to many studies [39, 40, 41, 42, 43] nanocurcumin decrease the levels of the liver enzymes by preserving the structural integrity of the cell membrane as shown in G4 compared to the other groups. In addition, ALT, AST, and ALP were significantly reduced by YCW, this result is compatible with many studies [44, 45], which explained the effect of yeast cell wall extract on liver enzymes activities. The significant decrease of AST and ALP in the G4 may be due to the free radical scavenging activity of nanocurcumin [43]. **El-Ghany et al. [46]**, **Gholami-Ahangaran et al. [47]**, and **Naseem et al. [48]** observed a significant decrease in total protein, albumin, and globulin in the aflatoxicated group compared to the group fed on basal diet only. This is indicative of the toxic effect of aflatoxin B1 on the liver and kidney and is an indicator of diminished protein synthesis [46]. As shown in our study, nanocurcumin and/or yeast cell wall extract that included in broiler diets improved the hepatic function as serum total protein, albumin, and globulin concentrations were increased and this is compatible with previous studies [49, 50, 51]. On the other hand, **Li et al. [52]** explained that YCW incorporation did not affect the same biochemical indices, including total protein, albumin, and globulin. Regarding results of the kidney function, many previous studies [53, 54, 55, 56] showed that the inclusion of aflatoxin in the diet led to a significant increase in the kidney function including urea, creatinine, and uric acid. In contrast, our result showed that the concentrations of the urea and uric acids were insignificantly increased in the G2 when compared with the other groups. On the other side, the creatinine concentration was significantly increased in the G2 when compared with G1 but non-significant when compared with the other groups.

Histopathological finding

Aflatoxin has a direct effect on the liver, which is a primary target organ. Similar to many studies [38, 57, 58] that reported the destructive effect of aflatoxin on hepatic tissues. The effect of nanocurcumin against AFB1 on the liver tissue histopathology comes in contrast with **Sayrafi et al. [14]** who investigated the protective effects of nanocurcumin on the liver toxicity induced by salinomycin in broiler chickens. Moreover, the photomicrograph of livers of broiler chicks fed aflatoxin with saccharomyces cell wall showed the same results as **Yalçın et al. [17]**. On the other side, the normal photomicrograph of the liver of the aflatoxin+

combination group in the present study may be due to the bio adsorbing feature of the functional ingredients in YCW (beta-glucan and mannan oligosaccharides) as reported by **Yiannikouris et al. [30]** and detoxification effect of curcumin nanoparticles as reported by **Yu & Huang [59]**.

Aflatoxin has been shown to destroy the normal morphology of the intestine [60]. However, there is a few reports on the effect of AFB1 on jejunal apoptosis [61]. Our result on the effect of aflatoxin on the jejunum section is compatible with **Peng et al. [61]**, **Jahanian et al. [5]**, and **Wang et al. [62]**. Yeast and yeast components, especially *Saccharomyces cerevisiae* cell walls, are recent approaches to eliminate side effects of AFB1 [63]. Moreover, **Reisinger et al. [64]** investigated the effect of Yeast derivatives (yeast cell wall fragments and yeast extract derived from *Saccharomyces cerevisiae*) on the jejunum of broiler chickens. They concluded that birds fed 0.1% and 0.2% Yeast derivative has a significantly higher density of goblet cells (number per 10 µm of villi length) than the control group.

Previous studies have shown that curcumin detoxification mechanisms, including inhibition of AFB1 phase I enzyme-mediated biotransformation and phase II enzyme activity up-regulation, can control the carcinogenic effects induced by AFB1 and the toxic effects attributed to the positive regulation of cells [65]. Therefore, the present study found that the photomicrograph of the intestine (jejunum section) of the aflatoxin + Nano group showed a mild degree of degenerative and desquamative changes within the intestinal villi with improvement of villi length compared to aflatoxin group similar to this study of **Rahmani et al. [34]**. Who found that villus height (VH), villus height/crypt depth ratio, and the villus surface area (VS) were higher in birds fed curcumin / nanocurcumin supplements than those fed control food. Furthermore, our study showed that photomicrograph of jejunum section of aflatoxin + combination group showed normal histology of intestinal villi with marked improvement of the thickness of mucosa. This is due to the bio adsorbing feature of the functional ingredients in YCW (beta-glucan and mannan oligosaccharides) [66] and the detoxification effect of curcumin nanoparticles [59].

Immunohistochemical study

The transcription nuclear factor-kappa B (NF- KB) consists of homo- and heterodimeric complexes formed from the Rel family of proteins. There are five members of the Rel/ NF- KB proteins in vertebral cells including p65 (Rel A), p50/p105, p52/100, c-Rel, and Rel B. The most common NF- KB is a heterodimer composed of p65 and p50 [67]. **Ma et al. [68]** reported AFB1 treatment increased the protein levels of NF-KB p65. Moreover, when **Fadl et al. [51]** studied the effect of dietary Mannan-oligosaccharide and β-Glucan (yeast cell wall extract) supplementation on hepatic transcripts of NF-KB of broiler challenged with *E. coli* O78, they found that the gene expression of NF KB was reduced and this indicates the protective effects and anti-inflammatory properties of YCW extract in broilers. This result is similar to the current result in spite of the current result obtained the expression of the NFkB P65 itself in the liver tissue of AF affected broilers. Moreover, the present result showed that nanocurcumin decreases the expression of NFkB P65 antibodies within the hepatocytes. This result is consistent with that obtained by **Chang et al. [69]** in the brain cell of rats. On the other side, the expression of the NFkB P65 in the liver tissue of the aflatoxin+ combination group was markedly decreased, which may be due to the anti-inflammatory and protective effects of the functional ingredients in YCW (beta-glucan and mannan oligosaccharides) [30] and detoxification effect of curcumin nanoparticles [59].

Expression of *CYP1A1* and *Nrf2* genes within the liver tissue of broilers:

Similar to the current result regarding the expression of the *CYP1A1* gene in the liver tissue of broiler chickens, **Zhang et al. [1]** found that there is a marked increase in the level of *CYP1A1* in the aflatoxin group compared with the control. As well, **Zhao et al. [38]** reported that AFB1 decreases *Nrf2* protein levels in broiler livers. **El-Gendy et al. [70]** reported that *Nrf2* protein levels significantly increased in aflatoxicated-bird supplemented with *Saccharomyces* cell wall. In addition, **Zaki et al. [71]** concluded that there was a decrease in *Nrf2* protein levels with the use of curcumin and nanocurcumin compared to that of deltamethrin-treated rats and this is what our study proves.

Aflatoxin residue within the liver and muscle tissue of broilers:

Residual AFB1 in the liver not only affects the performance and health of broiler chickens but also adversely affects the health of consumers of broiler products due to aflatoxin accumulation in the edible parts of poultry. Therefore, it is necessary to control the quality of poultry products and analyze aflatoxin residues in various poultry tissues taking into account public health and safety [19]. The result in the present study of the aflatoxin residue in G2 is in line with the findings of **Hussain et al. [36]**. Mycotoxins' harmful effects have been found to be prevented by glucan-based binders that adhere to them during digestion and inhibit their absorption from the gastrointestinal system [72]. Thus, the aflatoxin residue was decreased in the G3. On the other hand, **Limaye et al. [73]** reported that curcumin inhibits cytochrome P450 isoenzymes, notably the CYP2A6 isoform, lowering the generation of aflatoxin metabolites such as AFB1-8, 9-epoxide, and other toxic metabolites.

Conclusion

This trial introduces the new importance of nanocurcumin and/or *Saccharomyces* cell wall and new strategies to ameliorate the adverse effect of aflatoxin in broilers. Moreover, we advise by dietary supplementation of nanocurcumin and/or *Saccharomyces* cell wall may decrease the bad effect of many mycotoxins besides aflatoxin B1 because of their bio-adsorbing feature of the functional ingredients (beta-glucan and mannan oligosaccharides) in Yeast Cell Wall and the detoxification effect of curcumin nanoparticles.

Methods

Chemicals and treatments

Aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), total protein, albumin, creatinine, urea, and uric acids were purchased from Biodiagnostics Company (Dokki, Giza, Egypt). RNA extraction Kit was purchased from Introbio Company, Cat.No. 17063. Reverse transcriptase Polymerase chain reaction (RT-PCR) sensiFAST™ cDNA synthesis kit (Bioline, United Kingdom). All used primers are commercial base. Aflatoxin from Mycology and Mycotoxins Department, Animal Health Research Institute (ARC), Dokki, Egypt. Curcumin Nanoparticles (NT-Cur-NPs) were purchased from nanotech Egypt for photo-electronics at the city of 6 October, Al Giza. *Saccharomyces cerevisiae* cell wall extract (100%, Beta-glucan: 20% minimum & Manan Oligosaccharides: 20% minimum.) was obtained from IBEX Group for animal nutrition and health products and produced by Shandong Bio Sunkeen Co., Ltd (a state-owned high-tech enterprise that integrates R&D, production and sales with rich experience of yeast and nature yeast products).

Experimental birds, feeding, and design

One hundred and fifty one-day-old Cobb chicks with an average initial body weight of 55.6 g were obtained from a local hatchery. The birds were individually weighed, randomly allocated into 5 groups (30 chicks per group), and each group subdivided into three replicates (10 chicks per each). The basal diets were formulated in Table (7) according to **NRC [74]**. The experimental feeding was designed as follows:

Group 1: Basal diet

Group 2: Basal diet with 0.25 mg/kg AFB1 **[19]**

Group 3: Basal diet with 0.25 mg /kg AFB1+ Lancell YCW 1kg/ton according to Shandong Bio Sunkeen Co., Ltd & IBEX group

Group 4: Basal diet with 0.25 mg /kg AFB1 + NC 400 mg/kg **[34]**

Group 5: Basal diet with 0.25 mg /kg AFB1+ YCW 1kg/ton + NC 200 mg/kg **[34]**.

Housing and Management

The birds were reared in a clean, well-ventilated room, which was fumigated and disinfected with formaldehyde gas (obtained by mixing 40% formalin and potassium permanganate powder). The proper temperature of the room was obtained by using gas heaters and electric lamps (200 watts) in each partition were light/dark 23/1 hrs. /day. The floor of the room was divided into 15 divisions. Each compartment was covered with fresh clean straw forming a deep litter 4 centimeters deep. Each compartment was provided with an appropriate feeder and waterer.

Prophylaxis against the most common infectious diseases is carried out using Spectrama Vet (1 ml / 2 liters), Colistin sulfate (1g / 4 liters) for *Salmonellosis* & *E. coli* infections for the first three days, and Coxistac (0.1% of the diet) for coccidiosis. Chicks were vaccinated against Newcastle disease, IB, and Gumboro. After vaccination, chicks were given vitamin AD3E (1 ml/L drinking water) to improve chick vitality. Body weights (BW) of the chicks and feed intake were weighed at weekly intervals to calculate the other growth performance parameters.

Blood and serum samples

Birds were anesthetized on day 35 by intraperitoneal injection of sodium pentobarbital (50 mg/kg) to minimize suffering during blood sample collection. Blood samples (3ml/bird) were taken from vena brachialis under the wing of 5 birds in each group using 2 different capillary tubes for each one, EDTA tube for haematological evaluation **[75]** and another without anticoagulant for serum separation. Blood samples were clotted at room temperature. Serum separation was performed by centrifuging the coagulated blood at 3000 rpm for 15 minutes. Serum was collected and stored at -20°C for determination of urea, creatinine, albumin, total protein, uric acid, and activity of AST, ALT, and ALP using commercial kits, spectrophotometrically.

Tissue samples

The anesthetized broilers were sacrificed through cervical dislocation. After taking carcass and organs weight, sections of the liver, kidney, and intestine were immediately fixed in 10% formalin for histopathological

examination, also liver samples were taken and kept at -80°C for (RT-PCR) to determinate mRNA expression of cyp450 1A1 and Nrf2. To detect aflatoxin residue in the liver and muscle tissue, liver and muscle samples were collected from 5 birds of each group, also on day 50, liver and muscle samples were collected from the aflatoxin-fed group (G2) only and were stored in a deep freezer until use.

At the end, CO₂ suffocation was used to suffocate the remaining living birds in thick sacks. All of the birds (125), including dead and cervical dislocated birds, as well as samples and bedding material remnants, were buried in a hygienically regulated, well-constructed burial hole.

Carcass traits

Dressing percentage: five birds from each group were eviscerated, weighed without feather and head and the dressing percentage was calculated according to the following formula:

Dressing % = Dressed carcass weight/Live weight X 100.

Organs relative weight: Heart, gizzard, proventriculus, liver, spleen, thymus, bursa, and breast and leg muscle were weighed and their relative weights to body weight were calculated.

Histopathological examination

Following necropsy, liver and intestinal sections were obtained and promptly fixed in 10% buffered formalin before being processed for histological examination using conventional paraffin sections. **Bancroft & Gamble [76]** recommended cutting 5 m thick sections and staining them with hematoxylin and eosin (H&E).

QRT-PCR analysis

Reverse transcription-quantitative polymerase chain reaction (RT-qPCR) was used to analyze the gene expressions. Total RNA from the liver was extracted by using the TRIzol® reagent (Invitrogen, USA). Then, reverse-transcribing of the extracted RNA was done to create cDNA. Table (8) shows the employed primer sequences of the following; β -actin, CYP1A1, and Nrf2 genes. RT-qPCR reactions were completed by Power SYBR® Green PCR Master Mix (Applied Biosystems, USA). Reactions were performed by the 7500 Real-Time PCR System (Applied Biosystems, USA). Thermal cycles were performed at 95°C for 4 minutes, 40 cycles of 10 seconds at 95°C, 30 seconds at 60°C, and finally 10 seconds at 72°C. Data were presented as relative fold changes compared to the control's gene expressions.

Method for detection of aflatoxin residue in the liver and muscle tissue

The liver and muscle samples (5 samples/group) were taken after 35 days from the beginning of the experiment and after withdrawal of AFB1 from the diet of the aflatoxicated group (G2), (after the end of the experiment 2 weeks). The samples were stored in a deep freezer until used. According to **Schuller and Van Egmond [77]** the AFB1 residue was estimated in the liver and muscle samples by thin-layer chromatography (TLC).

Statistical analysis

The obtained data were statistically analyzed by one-way analysis of variance using spss to assess significant differences. A value of $P < 0.05$ was considered to be significant.

Declarations

Ethical approval

The current study was approved by the Ethical Committee for live birds sampling at the Faculty of Veterinary Medicine, Alexandria University, Egypt (0106699).

Guidelines

All methods were carried out in accordance with relevant guidelines and regulations.

ARRIVE guidelines

The authors confirm that the study was carried out in compliance with the ARRIVE guidelines

Consent for publication

Not applicable

Availability of data and materials

The datasets supporting the conclusions of this article are included within the article (and its additional files)

Competing interests

The authors declare that they have no competing interests

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Authors' Contributions

All authors contributed equally to this work whereas they designed, conducted the experiment and wrote the manuscript. All authors have read and approved the manuscript

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Tables

Table (1): Effect of dietary aflatoxin, Nano curcumin, and/or saccharomyces cell wall supplementation on the performance of broilers

Groups	G1	G2	G3	G4	G5
Items					
Initial weight (g)	55±1.50	57±1.50	57±0.90	56±1.60	55.4±1.11
Final weight (g)	2385±3.495 ^a	1605±2.79 ^e	2038 ±3.81 ^d	2097 ±2.91 ^c	2110±2.24 ^b
BWG (g)	2329.80±4.13 ^a	1548.00±3.26 ^e	1980.60±4.12 ^d	2042.30±3.72 ^c	2054.60±2.63 ^b
Feed intake (g)	3460±0.58 ^a	2800±0.00 ^d	3100±0.58 ^b	3089±0.57 ^c	3100±0.00 ^b
FCR	1.5±0.011 ^c	1.81 ± 0.004 ^a	1.57±0.004 ^b	1.51±0.002 ^c	1.51±0.003 ^c

Values are means ± standard error. Mean values with different letters at the same row significantly differ at ($P \leq 0.05$). G1= group fed control diet without supplement; G2= group fed control diet with aflatoxin; G3=group fed control diet with aflatoxin and saccharomyces cell wall; G4=group fed control diet with Nano curcumin; G5=group fed control diet with saccharomyces cell wall and Nano curcumin.

Table (2): Effect of dietary aflatoxin, Nano curcumin, and/or saccharomyces cell wall supplementation on some carcass traits of broiler as percent of live body weight

Groups	G1	G2	G3	G4	G5
Items %					
Dressing	76.69 ± 3.48 ^{ab}	68.46 ± 2.42 ^c	73.25 ± 0.31 ^b	76.45 ± 0.69 ^{ab}	80.52 ± 1.32 ^a
Breast muscle	45.32 ± 2.48 ^b	43.59 ± 0.17 ^b	46.54 ± 1.29 ^{ab}	46.78 ± 1.24 ^{ab}	50.11 ± 1.23 ^a
Leg muscle	31.37 ± 1.01 ^a	24.78 ± 0.68 ^b	30.41 ± 0.63 ^a	29.67 ± 0.56 ^a	29.66 ± 0.38 ^a
Liver	2.19 ± 0.12 ^b	3.94 ± 0.87 ^a	1.57 ± 0.19 ^b	1.86 ± 0.09 ^b	1.53 ± 0.05 ^b
Gizzard	1.50 ± 0.22	1.41 ± 0.09	1.58 ± 0.13	1.60 ± 0.08	1.33 ± 0.13
Proventriculus	0.26 ± 0.01	0.27 ± 0.03	0.26 ± 0.03	0.27 ± 0.02	0.27 ± 0.01
Thymus	0.10 ± 0.02 ^b	0.17 ± 0.05 ^{ab}	0.19 ± 0.02 ^a	0.11 ± 0.02 ^{ab}	0.12 ± 0.02 ^{ab}
Spleen	0.09 ± 0.03 ^{ab}	0.15 ± 0.03 ^a	0.05 ± 0.00 ^b	0.06 ± 0.01 ^b	0.04 ± 0.00 ^b
Bursa	0.12 ± 0.02	0.19 ± 0.07	0.09 ± 0.01	0.09 ± 0.00	0.09 ± 0.02

Values are means ± standard error. Mean values with different letters at the same row significantly differ at ($P \leq 0.05$). G1= group fed control diet without supplement; G2= group fed control diet with aflatoxin; G3=group fed control diet with aflatoxin and saccharomyces cell wall; G4=group fed control diet with Nano curcumin; G5=group fed control diet with saccharomyces cell wall and Nano curcumin.

Table (3): Effect of dietary aflatoxin, Nano curcumin, and/or saccharomyces cell wall supplementation on hematological parameters of broiler.

Groups	G1	G2	G3	G4	G5
Items %					
RBCs count ($\times 10^6/\mu\text{l}$)	3.57 ± 0.17^a	2.30 ± 0.18^c	3.30 ± 0.10^{ab}	3.13 ± 0.04^b	3.43 ± 0.15^{ab}
Hb (g/dl)	11.07 ± 0.75	9.36 ± 0.85	9.89 ± 0.29	9.38 ± 0.11	10.29 ± 0.44
HCT (%)	41.92 ± 1.06^a	29.55 ± 0.48^c	39.55 ± 1.15^{ab}	37.52 ± 0.44^b	41.16 ± 1.78^a
WBCs count ($\times 10^3/\mu\text{l}$)	20.12 ± 1.00^a	13.57 ± 1.31^b	16.52 ± 0.89^{ab}	18.46 ± 1.72^a	18.63 ± 1.09^a
LYM ($\times 10^3/\mu\text{l}$)	62.49 ± 1.86^a	40.25 ± 1.08^b	55.78 ± 2.56^a	59.14 ± 3.95^a	59.81 ± 3.75^a
HET ($\times 10^3/\mu\text{l}$)	32.76 ± 1.75^b	50.70 ± 0.92^a	37.63 ± 2.01^b	35.71 ± 3.48^b	35.80 ± 3.90^b
ESI ($\times 10^3/\mu\text{l}$)	1.17 ± 0.09^c	2.14 ± 0.09^a	1.54 ± 0.03^b	1.27 ± 0.09^c	1.15 ± 0.02^c
MON ($\times 10^3/\mu\text{l}$)	3.59 ± 0.28^c	6.92 ± 0.42^a	5.05 ± 0.55^b	3.87 ± 0.42^{bc}	3.24 ± 0.21^c

Values are means $\pm$ standard error. Mean values with different letters at the same row significantly differ at ($P \leq 0.05$). G1= group fed control diet without supplement; G2= group fed control diet with aflatoxin; G3=group fed control diet with aflatoxin and saccharomyces cell wall; G4=group fed control diet with Nano curcumin; G5=group fed control diet with saccharomyces cell wall and Nano curcumin. RBCs = red blood cells, Hb = hemoglobin, HCT = hematocrit, WBCs = white blood cells, LYM = lymphocyte, HET = heterophil, ESI = eosinophil, MON = Monocytes, BAS = basophil.

Table (4): Effect of dietary aflatoxin, Nano curcumin, and/or saccharomyces cell wall supplementation on some biochemical parameters of broiler.

Groups	G1	G2	G3	G4	G5
Items					
ALT (U/L)	25.30 ± 2.48 ^c	39.00 ± 2.08 ^a	31.37 ± 0.58 ^b	19.00 ± 2.31 ^d	16.60 ± 0.17 ^d
AST (U/L)	20.00 ± 1.73 ^e	138.33 ± 2.03 ^a	66.67 ± 2.19 ^b	36.10 ± 0.84 ^d	58.50 ± 0.87 ^c
ALP (U/L)	931.67 ± 2.73 ^b	1197.33 ± 1.76 ^a	911.33 ± 1.86 ^c	518.00 ± 3.61 ^e	537.00 ± 1.53 ^d
T. Protein (g/dl)	6.07 ± 0.12 ^b	3.60 ± 0.21 ^c	6.33 ± 0.12 ^b	7.27 ± 0.19 ^a	7.03 ± 0.15 ^a
Albumin (g/dl)	3.60 ± 0.18 ^a	2.81 ± 0.41 ^b	3.54 ± 0.04 ^a	3.71 ± 0.12 ^a	3.81 ± 0.03 ^a
Globulin (g/dl)	2.47 ± 0.06 ^d	0.79 ± 0.08 ^e	2.79 ± 0.08 ^c	3.55 ± 0.07 ^a	3.23 ± 0.12 ^b
Creatinine (mg/dl)	0.32 ± 0.01 ^b	0.44 ± 0.03 ^a	0.38 ± 0.02 ^{ab}	0.38 ± 0.02 ^{ab}	0.38 ± 0.04 ^{ab}
Urea (mg/dl)	11.31 ± 0.66	12.23 ± 0.39	11.44 ± 0.68	11.55 ± 0.16	12.05 ± 0.65
Uric acid (mg/dl)	5.69 ± 0.46	6.92 ± 0.92	5.51 ± 0.51	5.76 ± 0.53	6.23 ± 0.77

Values are means ± standard error. Mean values with different letters at the same row significantly differ at ($P \leq 0.05$). G1= group fed control diet without supplement; G2= group fed control diet with aflatoxin; G3=group fed control diet with aflatoxin and saccharomyces cell wall; G4=group fed control diet with Nano curcumin; G5=group fed control diet with saccharomyces cell wall and Nano curcumin.

Table (5): Effect of dietary aflatoxin, Nano curcumin, and/or saccharomyces cell wall supplementation on intestinal morphometrical data of intestinal sections of broiler.

Groups	G1	G2	G3	G4	G5
Items					
Villi length (µm)	1666.85 ± 71.21 ^c	680.75 ± 34.82 ^a	985.99 ± 32.90 ^b	1153.26 ± 24.13 ^d	1428.94 ± 19.97 ^d
Villi width (µm)	119.02 ± 5.29 ^e	246.43 ± 4.72 ^a	164.74 ± 14.83 ^b	128.27 ± 15.72 ^d	97.23 ± 13.73 ^c
Crypt depth (µm)	223.55 ± 8.82 ^b	136.16 ± 13.69 ^a	167.12 ± 7.75 ^c	175.98 ± 5.30 ^e	201.22 ± 6.94 ^d
Goblet cell (No/mm ²)	208.74 ± 4.24 ^b	117.89 ± 8.82 ^c	195.49 ± 7.57 ^b	185.15 ± 3.40 ^a	235.41 ± 4.96 ^a
Crypt/Villi ratio	7.49 ± 0.49 ^a	2.77 ± 0.19 ^b	5.93 ± 0.29 ^a	6.56 ± 0.11 ^a	7.12 ± 0.19 ^a

Values are means ± standard error. Mean values with different letters at the same row significantly differ at (P≤0.05). G1= group fed control diet without supplement; G2= group fed control diet with aflatoxin; G3=group fed control diet with aflatoxin and saccharomyces cell wall; G4=group fed control diet with Nano curcumin; G5=group fed control diet with saccharomyces cell wall and Nano curcumin.

Table (6): Effect of dietary aflatoxin, Nano curcumin, and/or saccharomyces cell wall supplementation on aflatoxin residues in the liver and muscle tissues of broilers

Groups	G1	G2	G3	G4	G5
Items					
Residue in the liver tissue at the end of the experiment (µg/kg)	0.0±0.0 ^c	13.13 ± 0.32 ^{aX}	2.90 ± 0.30 ^b	0.0±0.0 ^c	0.0±0.0 ^c
Residue in the liver tissue at 2 weeks after the end of the experiment (µg/kg)	-	0.80 ± 0.20 ^Y	-	-	-
Residue in the muscle tissue at the end of the experiment (µg/kg)	0.0±0.0 ^c	5.77 ± 0.25 ^a	2.17 ± 0.21 ^b	0.0±0.0 ^c	0.0±0.0 ^c

Values are means ± standard error. Mean values with different letters at the same row (a-c) and same column (X-Y) significantly differ at (P≤0.05). G1= group fed control diet without supplement; G2= group fed control diet with aflatoxin; G3=group fed control diet with aflatoxin and saccharomyces cell wall; G4=group fed control diet with Nano curcumin; G5=group fed control diet with saccharomyces cell wall and Nano curcumin.

Table (7): Ingredients and calculated chemical composition of the used basal diets.

Ingredients	Ingredients%		Chemical composition		
	Starter	Grower and finisher	Items	Starter	Grower and finisher
Yellow Corn	54.5	60	ME (Kcal/Kg)	3201.5	3221
Soybean (44%)	27.27	20	Crud protein%	23	20.08
Corn gluten meal (62%)	10	8.73	Calcium %	1	0.9
Wheat bran	0	3.5			
Sunflower oil	4	4	Available phosphorus%	0.45	0.35
Dicalcium phosphate	1.78	1.3	Lysine%	1.1	1
Limestone	1.3	1.4	Meth. + Cyst%	0.9	0.72
Lysine	0.13	0			
DL-Methionine	0.1	0.2			
Common salt	0.4	0.4			
Choline chloride (60%)	0.22	0.17			
Premix*	0.3	0.3			

*The used premix (*Multivita Co.*) composed of vitamin A 12000000 IU, vitamin D₃ 2200000 IU, vitamin E 10000 mg, vitamin K₃ 2000 mg, vitamin B₁ 1000 mg, vitamin B₂ 5000 mg, vitamin B₆ 1500 mg, vitamin B₁₂ 10 mg, Niacin 30000 mg, Biotin 50 mg, Folic acid 1000 mg, Pantothenic acid 10000 mg, Iron 30000 mg, Manganese 60000 mg, Copper 4000 mg, Zinc 50000 mg, Iodine 1000 mg, Cobalt 100 mg, Selenium 100 mg, calcium carbonate (CaCO₃) carrier to 3000g

Table (8) Primers used for qRT-PCR analysis

Gene	Gene bank No.	Forward primers Sequence (5'-3')	Reverse primers Sequence (5'-3')	Product size (bp)
β-actin	L08165	ATGGCTCCGGTATGTG C AA	TGTCTTTCTGGCCCATACCAA	178
<i>CYP1A1</i>	X99454.1	CACTTTCTGCCTGCTCCTG	GGTCCTTCCTCAGCTCCAG	125
<i>Nrf2</i>	NM_001030756.1	CTGCTAGTGGATGGCGAGAC	CTCCGAGTTCTCCCCGAAAG	132

Figures

Figure 1

Photomicrograph of hepatic tissues of different treated groups stained with hematoxylin and eosin (H&E) where (A) G1: showing normal histology in which the hepatocytes (H) and portal area (PA) consisting of bile duct, portal vein, hepatic arteriole brunch, (B) G2: showing fibrosis of the portal area (PA) with newly formed bile ducts (arrow) and mononuclear cells infiltration within the portal area (arrow head) beside wide periportal hepatocytic cells necrosis (H), (C) G3: showing the hepatocytes (H) with multifocal mild cytoplasmic sharp outlines vacuoles referring to fatty change and normal portal area (PA), (D) G5: showing normal histology including the hepatocytes (H) and portal area (PA) consisting of bile duct, portal vein, hepatic arteriole brunch,

(E) G4: showing normal hepatocytes (H) and portal area (PA) slightly infiltrated with mononuclear cells, (F) showing quantities scoring of the hepatic lesions in different groups, bar= 50 μ m.

Figure 2

Photomicrograph of intestine (jejunum section) of different treated groups stained with hematoxylin and eosin (H&E) where (A) G1: showing normal histology of intestinal villi, (B) G2: showing mucosal necrosis of the intestinal villi with presence of necrotic core associated with blunting and villous atrophy, (C) G3: showing marked decrease of mucousal atrophy with an increase of villi length, (D) G4: showing mild degree of

degenerative and desquamative changes within the intestinal villi with improvement of villi length, (E) G5: showing normal histology of intestinal villi with marked improvement of the thickness of mucosa, bar= 500 μ m.

Figure 3

Liver of the different treated groups where (A) G1: showing mild immunostaining of NFkB P65 antibody within the hepatocytes (arrowheads), (B) G2: showing marked cytoplasmic and nuclear expression of NFkB P65 antibody within the hepatocytes (arrowheads), NFkB P65 IHC, (C) G3: showing decrease the expression of

NFkB P65 antibody within the hepatocytes (arrowheads), (D) G4: showing decrease the expression of NFkB P65 antibody within the hepatocytes (arrowheads), NFkB P65 IHC, (E) G5: Liver of of aflatoxin+ combination group showing marked decrease the NFkB P65 expression within the hepatocytes (arrowheads), (F) showing percentage of NFkB P65 positive cells in different groups, NFkB P65 IHC, X200, bar= 40 μ m. B: nfkb p65 labelling index

Figure 4

(A) Expression of *CYP 1A1* G5ne within the liver tissue, (B) Expression of *Nrf2* G5ne within the liver tissue. Data are expressed as mean $\pm$ SD, and superscript samples # and ** indicate significance in comparison with the control group.

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