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PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

OPEN ACCESS Pakistan Journal of Biological Sciences

ISSN 1028-8880
DOI: 10.3923/pjbs.2021.1091.1102

Research Article
Hepatoprotective Role of Clay and Nano Clay for Alleviating Aflatoxin Toxicity in Male Rats

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Abstract

Background and Objective: Aflatoxin formed by *Aspergillus* sp. causes acute hepatotoxicity by DNA damage, gene expression disruption and induced liver carcinoma in humans and laboratory animals. The objectives of this research were to evaluate the protective role of both clay and nano clay as adsorbents to inhibit the side effect of Aflatoxin (AF) by measures the common biological assay of aflatoxicosis in rats along with hepatic gene expression and comet assay. **Materials and Methods:** Six weeks old male albino rats were distributed into 6 groups with 10 rats per group fed on, Group 1: Basal diet, Group 2: Basal diet with clay (5 g kg^{-1} diet), Group 3: Basal diet with nano clay (5 g kg^{-1} diet), Group 4: AF-contaminated diet (1 mg kg^{-1} diet), Group 5: AF with clay, Group 6: AF with nano clay. **Results:** AF induced a noticeable increase in the liver function parameters, accompanied by a significant decrease in antioxidant enzyme activities and significant histological alterations in liver tissues. The obtained qPCR results showed a significant up regulation in the expression of Cyp3A6, HO-1, TNFα and NFKB genes in the liver of rats treated with aflatoxin. In contrast, there is a significant down regulation in the expression levels of the Glut2 gene in liver rats treated with aflatoxin. Also, aflatoxin induced a significant increase in DNA damage. Clay and nano clay succeeded in ameliorating the toxic effects of aflatoxin. **Conclusion:** The results indicated the effective role of clay and nano clay in alleviating aflatoxin and reduce its harmful effects.

Key words: Aflatoxin, clay, nano clay, gene expression, DNA fragmentation, immunosuppression, aspartate aminotransferase

Citation: Amany, A. El-Sadawy, Amina, M.G. Zedan and H.M.G. El-Dein, 2021. Hepatoprotective role of clay and nano clay for alleviating aflatoxin toxicity in male rats. Pak. J. Biol. Sci., 24: 1091-1102.

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Aspergillus flavus and *Aspergillus parasiticus* are two important moulds that produce Aflatoxin (AF)[1]. The contaminated food and feed, particularly grains and nuts, in tropical countries, particularly Sub-Saharan Africa and Southeast Asia, under pre-or post-harvest conditions[2]. The most common and dangerous Aflatoxin is Aflatoxin B1 (AFB1) and the liver is the target organ for it [3,4]. The exposure to AFB1 resulted in growth stunting, immunosuppression, mutagenicity, genotoxicity, increasing hepatocellular carcinoma incidence in animals and humans[5-7] Aflatoxicosis (toxic effects of aflatoxin) cases are characterized by direct liver damage, hemorrhagic necrosis of liver and liver cancer[8]. Aflatoxin's harmful effects in domestic poultry increased activity of various enzymes aspartate aminotransferase (AST) and Gamma-Glutamyl Transferase (GGT)[9]. The genotoxic effects of AF are intimately linked with its biotransformation through the cytochrome P450 to the highly reactive AFB1-exo-8, 9-epoxide, which can form adducts with the DNA[10] and produce Reactive Oxygen Species (ROS)[11,12]. These adducts could obstruct transcription and translation, producing hepatotoxicity by disrupting the control of functional gene expression[4].

Clay minerals such as montmorillonite, bentonite and clinoptilolite are abundant and environmentally friendly[13]. They effectively adsorb the mycotoxins in the alimentary tract of animals and decrease their bioavailability[14]. Clay minerals are as an anticaking agent for animal feed, have been reported to prevent diseases associated with aflatoxicosis in animals, including chicks, turkey poults and pigs[15,16]. Abbes *et al.*[17] reported that the addition of montmorillonite (the main compound of bentonite) reduced AFB1 related death in a colon-cancer cell line *in vitro*. Moreover, Nones *et al.*[18] demonstrated that the co-incubation of AFB1 and clays was effective at reducing the toxicity induced by mycotoxins in Caco-2 cells. Protective effects against AFB1 were also reported by Turkez and Sisman[19], where hydrated sodium calcium aluminosilicate prevented the adverse effects induced by AFB1 in human lymphocytes. Clay modification using nanotechnological methods makes it possible to change or enhance their properties, providing great opportunities for the development of new high-effective materials.

The objectives of this research were to evaluate the protective role of both clay and nano clay as adsorbents to ameliorate the effect of aflatoxin by measuring the common biological assay of aflatoxicosis in rats along with hepatic gene expression and comet assay.

MATERIALS AND METHODS

Study area: The study was carried out at the Department of Biological and Environmental Sciences, Faculty of Home Economics, Al- Azhar University from March-May, 2019.

Aflatoxins production: The aflatoxin was produced by the fermentation of ground maize by *Aspergillus flavus* as mention previously[20]. The fermented maize was ground to a fine meal and the AF concentration was measured by HPLC[21]. The AF within the maize meal consisted of 94.6% B_1, 5.4% B_2, 0% G_1 and 0% G_2 based on total AF (156.84 ppm) in the maize powder (Table 1). The maize meal was added into the basal diet to give the appropriate level of 1 mg of total AF/kg diet.

Clay and nano clay samples: Bentonite was supplied by Misr Nanotechnology Co. (Alexandria, Egypt). For preparation nano clay the bentonite powder is pulverized using standard ceramic method[22]. The time taken for milling sample is 8 hrs and kept in an airtight container until characterization and analysis. The size of particles was determined by a Transmission Electron Microscope (TEM) and the size of nanoparticles was 43.1, 39.8, 39.3, 37.6, 32.1 (Fig. 1).

Experimental animals: Sixty male albino rats (Sprague Dawley strain) were obtained from the Laboratory Animal Colony, Helwan, Cairo-Egypt and weighed 100±10 g. Rats were kept in clean cages, given 12 hrs light and dark cycles and had unlimited access to food and clean tap water. All rats received a basal diet for a week before the start of the experiment for adaptation and to ensure normal growth and behaviour. Before the experiment began, the animals were given a week to acclimate to their new surroundings. Animals were divided to 6 groups (10 rats/group) and were maintained on their respective diet for eight weeks as follow:

- Group 1 (G_1): Animals fed on basal diet as control
- Group 2 (G_2): Control+(5 g kg^{-1} diet) clay
- Group 3 (G_3): Control+(5 g kg^{-1} diet) nano clay
- Group 4 (G_4): AF+(1 mg kg^{-1} diet)
- Group 5 (G_5): AF+(5 g kg^{-1} diet) clay
- Group 6 (G_6): AF+(5 g kg^{-1} diet) nano clay

Throughout the experiment, the animals were monitored daily for signs of toxicity. At the end of the experiment, all of the animals were given a 12 hrs fast. Under diethyl ether anaesthesia, eight from each group were sacrificed and then blood samples were collected from the hepatic portal vein.

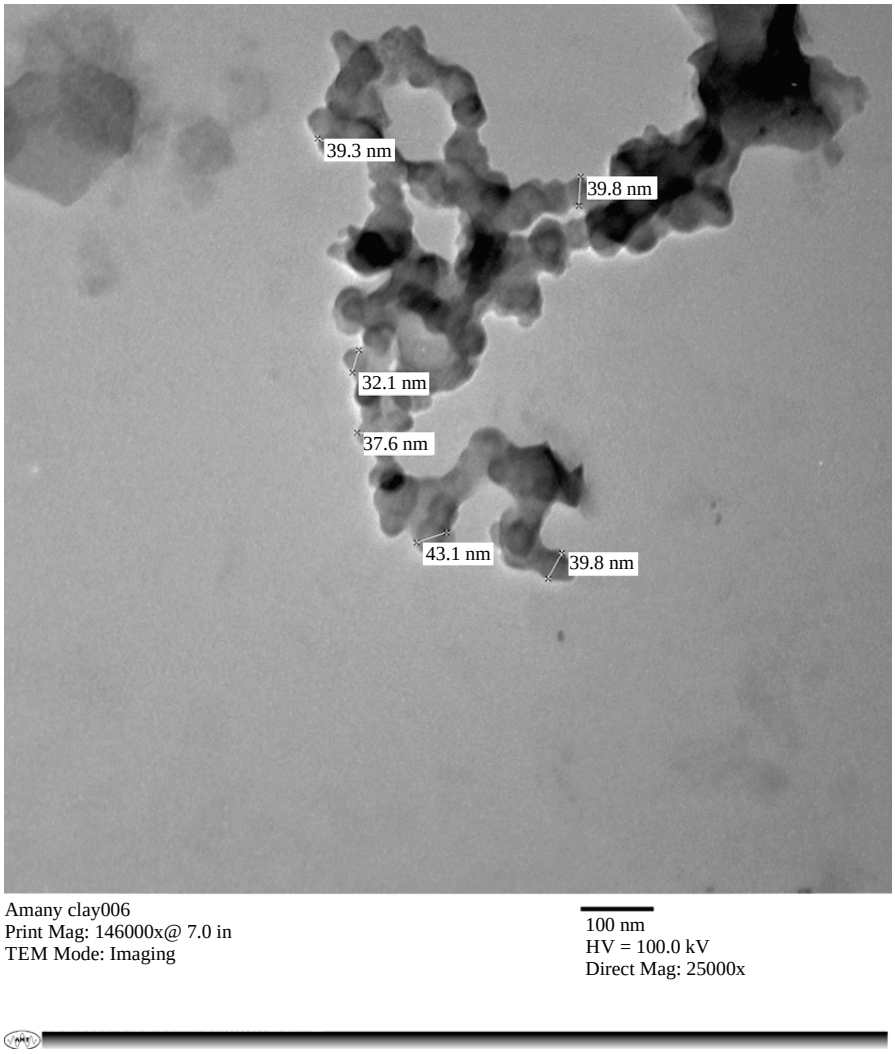

Fig. 1: Scanning electron microscopy to the particle size of nano clay, it was range 32.1-43.1 nm

Table 1: Concentrations of aflatoxin type in the sample

Aflatoxin type	Concentration (μg kg^{-1})
B_1	148.37
B_2	8.47
G_1	ND
G_2	ND
Total	156.84

After the collection of blood samples, serum was separated under cooling centrifugation and kept at -20 until analysis. The serum was used for the determination of ALT, AST and GGT according to the kits instructions using Jenway spectrophotometer 6715 (Staffordshire, UK), GPX, SOD and CAT according to the kits instructions. The organs were immediately removed and weighed, a sample of the liver of each animal was dissected and kept in liquid nitrogen then under -80 for molecular analysis.

Histopathology analysis: Other samples of the liver from all rats were fixed in 10% neutral formalin and paraffin-embedded. Sections (5 μm thickness) were stained with hematoxylin and eosin (H and E) for the histological examination[23].

Molecular analysis

Determination of gene expression with real-time PCR in hepatic tissue: The RNeasy kit (Qiagen) is used in the isolation of total RNA from the liver as described previously[24]. The purity and integrity of RNA were assessed by Nanodrop and 1% agarose gels electrophoresis, respectively. The Quantiscript reverse transcriptase is used in RNA reverse transcription to cDNA. Real-time PCR reaction contains cDNA as a template in the presence of QuantiTect SYBR Green qPCR Master Mix and

Table 2: Forward and reverse primers sequence for TNFα, Cyp3A6, HO-1, NFκB, Glut2 and β-actin genes

Gene	Forward primer ('5 ------ '3)	Reverse primer ('5 ------ '3)
TNFα	GCATGATCCGCGACGTGGAA	AGATCCATGCCGTTGGCCAG
Cyp3A6	TCCTTCATTATGCATTTGTTGGCC	ACCACCATGTCCAGATATTCCATC
HO-1	CCTCCCTGTACCACATCTACGT	AGCTCCTCCGGGAAGTAGAG
NFkB	TCTGTTTCCCCTCATCTTTCC	TGGGTGCGTCTTAGTGGTAT
Glut2	TAGTCAGATTGCTGGCCTCAGCTT	TTGCCCTGACTTCCTCTTCCAACT
β-actin	TCCTTCCTGGGCATGGAGTC	GGATGTCCACGTCGCACTTC

gene-specific primers, designed by the Primer 3 web-based tool based on the published rat sequence (Table 2), along with Step One Plus real-time PCR system (Applied Biosystem, USA) and reaction cycles as described by[25]. The Critical threshold (Ct) quantities for the target genes were normalized with quantities of the Ct of the internal control (β-actin)[26].

Comet assay: Comet assay was performed according to Eldamaty *et al.*[27]. In brief, a weight of 1 g of crushed samples was transferred to 1 mL ice-cold PBS. This suspension was mixed for 5 min then filtered.100 µL of cell suspensions were integrated with 600 µL of agarose (low-melting, 0.8% in PBS). This mixture (100 µL) was spread on slides. The slides were soaked in lysis buffer for 15 min. They were then placed in the electrophoresis chamber having the same lysis buffer without SDS. The conditions of electrophoresis were 100 mA and 2 V/cm for 2 min. staining with ethidium bromide. A fluorescent microscope was used to examine the DNA fragment migration patterns at a magnification of 40×s and with an excitation filter of 420-490 nm. Software of komet five image analysis that established by Ltd (Kinetic Imaging). (Liverpoo1, UK) attached to a CCD camera. It is used to assess the quantitative and qualitative extent of DNA damage by calculating the DNA migration length and the percentage of migrated DNA.

Statistical analysis: All the data were reported as means±SD. The statistical significance was evaluated by one-way ANOVA (analysis of variance) using SPSS, 20 software and the individual comparisons were obtained by Duncan's multiple range test. Values were found statistically significant when $p<0.05$.

RESULTS

Effect of clay and nano clay on liver function in rats treated with aflatoxin: The results of the current study revealed that various liver enzymes were seriously affected by AF treatment. The results in Table 3 showed a marked increase in the liver enzymes (AST, ALT and GGT) that induced by aflatoxin with diet. This increase was significantly decreased with the treatment by clay and nano clay in both ALT and GGT but this decrease was in the normal range. To AST levels the aflatoxin treatment with nano clay didn't reduce the high level of the enzyme although these increase in the normal range.

Effects of feeding aflatoxin-contaminated diets with or without clay and nano clay on the antioxidant activity in rats: There was a significant reduction in antioxidants (CAT, GPX and SOD) in the treatment with aflatoxin compare to other groups without aflatoxin but this decrease in GPX in the normal range. On the other hand, the treatment with clay or nano clay plus AFB1 resulted in significant improvements in all the tested parameters toward the control levels (Table 4).

Effect of aflatoxin, clay and nano clay treatments on Cyp3A6, HO-1, TNFα, NFKB and Glut2 gene expression in rat liver: The biochemical results were confirmed by the gene expression for some genes involved in aflatoxin toxicity in the liver tissue. The obtained qPCR results showed a significant up-regulation in the expression levels of Cyp3A6, HO-1, TNFα and NFKB genes in the liver of rats treated with aflatoxin (G_4) as compared to other, negative control group (G_1), (G_2), (G_3) (Fig. 2a-d). This elevated expression was significantly down-regulated following by (G_5) and (G_6), the lowest expression with rats fed AF+nano clay. However, the expression remained higher compare to groups (G_1-G_3). Also, no significant differences were noticed with the groups (G_1-G_3) in Cyp3A6, HO-1 and TNFα genes (Fig. 2a-c). On the other hand, obtained qPCR results showed significant downregulation in the expression levels of the Glut2 gene in the liver of rats treated with aflatoxin (G_4) compared to other treatments and control groups (Fig. 2e). Administration Aflatoxin with clay and nano clay upregulate the gene expression of Glut2. However, the expression remained lowest compare to groups (G_1-G_3) (Fig 2e).

Effect of aflatoxin, clay and nano clay treatments on DNA fragmentation in rats liver: A comet assay was performed to assess DNA damage in the liver. The results of the comet assay were shown in (Fig. 3a-f) and Table 5. These results showed that aflatoxin (Fig. 3d) induced a marked increase in DNA

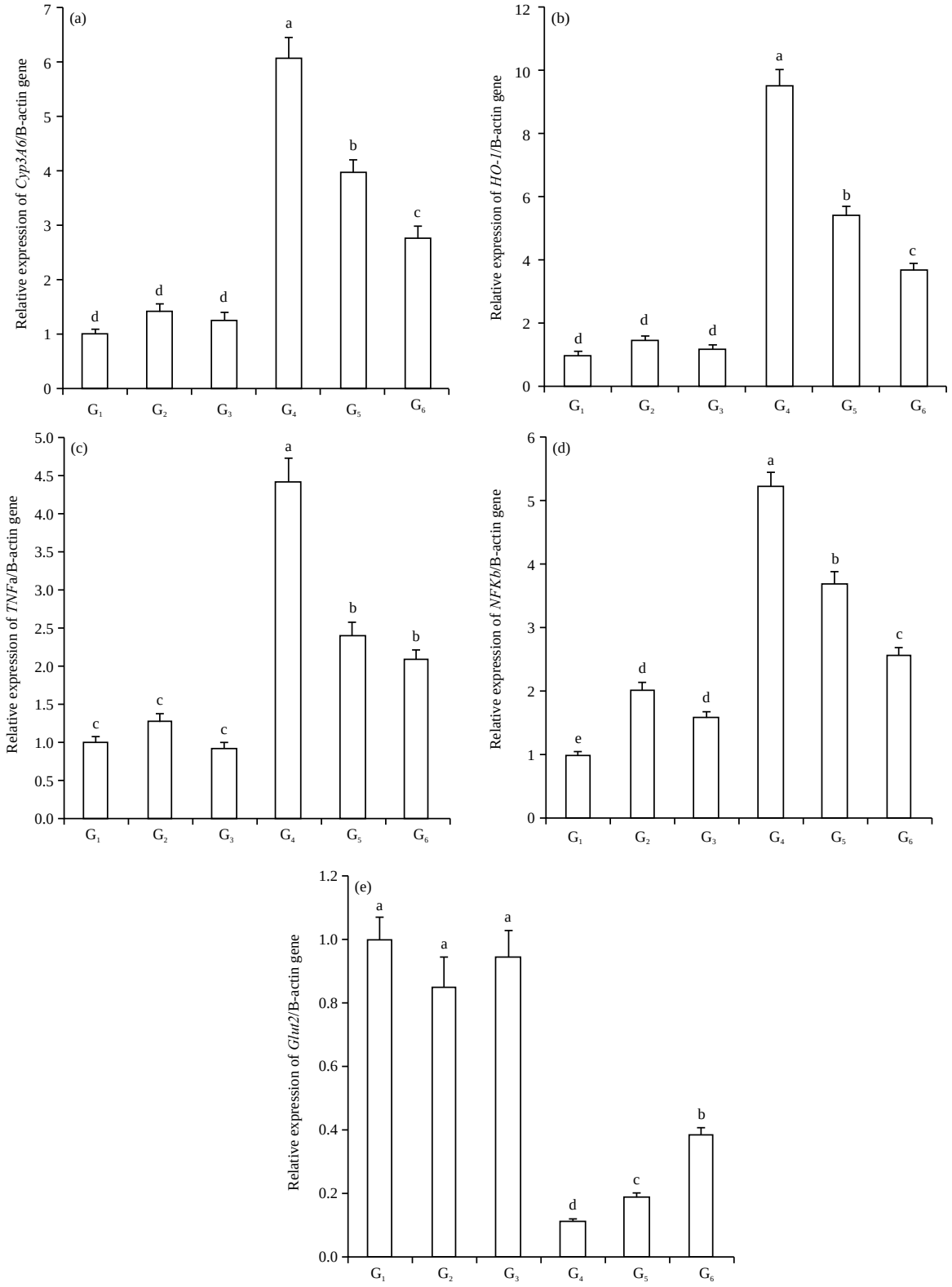

Fig. 2(a-e): Effect of aflatoxin, clay and nano clay treatments on Cyp3A6 (A), HO-1(B), TNFα (C), NFKB (D) and Glut2 (E) gene expression in rat liver

Data presented as fold change Mean±SEM (n = 7/group). Columns carrying different letters are significantly different at p<0.05

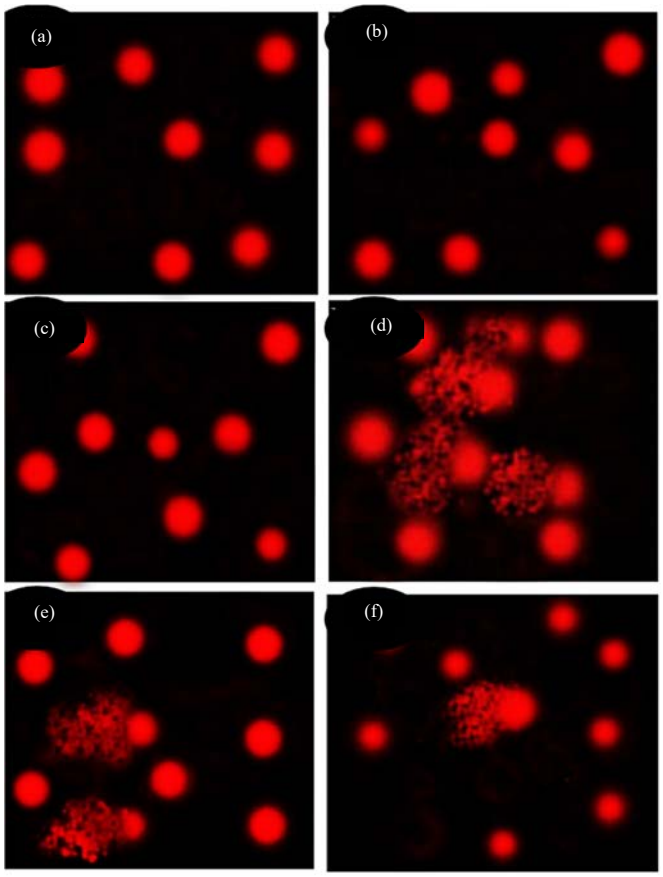

Fig. 3(a-f): Photomicrographs representation of DNA damage, using comet assay, in rat liver

Negative control group (A), clay+control group (B), nano clay+control group (C), aflatoxin group (D), aflatoxin with clay treated group (E) and aflatoxin with nano clay treated group (F)

Table 3: Effect of clay and nano clay on liver function in rats treated with aflatoxin

Treatments	AST (U L^{-1})	ALT (U L^{-1})	GGT (U L^{-1})
G_1	108.30±6.56^b	53.33±11.8^b	27.50±2.81^b
G_2	101.60±8.7^b	48.40±3.84^b	31.00±1.58^b
G_3	95.40±13.59^b	54.00±13.74^b	29.00±2.12^b
G_4	130.80±6.6^a	74.00±4.58^a	43.75±4.11^a
G_5	104.60±13.35^b	45.60±7.70^b	35.00±12.9^b
G_6	126.00±9.97^a	51.00±13.03^b	31.83±6.67^b
Sig.	0.000	0.026	0.011
Normal range	74-143	18-45	9 -85

G_1 (control): G_2 (control+clay): G_3 (control+nano clay): G_4 (AF): G_5 (AF+clay); G_6 (AF+nano clay); AST: Aspartate aminotransferase; ALT: Alanine Aminotransferase. GGT: Gamma-glutamyltransferase. Within each column, means superscript with different letters are significantly different ($p<0.05$)

Table 4: Effects of feeding aflatoxin-contaminated diets with or without clay and nano clay on the antioxidant activity in rats

Treatments	GPX (μmol L^{-1})	SOD (U mg^{-1})	CAT (U mg^{-1})
G_1	1.10±0.10b^a	1.56±0.40^b	82.00±1.00^a
G_2	1.40±0.40^a	1.40±0.17^b	79.96±0.04^b
G_3	0.82±0.06^{ab}	1.61±0.38^b	85.72±1.27^a
G_4	0.50±0.10^c	0.60±0.10^c	46.66±5.77^c
G_5	0.56±0.06^c	1.64±0.84^b	82.25±1.65^a
G_6	1.22±0.07^a	2.82±0.32^a	80.63±0.48^b
Sig.	0.005	0.000	0.002
Normal range	0.21-1.5	0.71- 3.7	80-85

G_1 (control): G_2 (control+clay): G_3 (control+nano clay): G_4 (AF): G_5 (AF+clay): G_6 (AF+nano clay); SOD: Superoxide dismutase, CAT: Catalase, GPx: Glutathione peroxidase, Within each column, means superscript with different letters are significantly different ($p<0.05$)

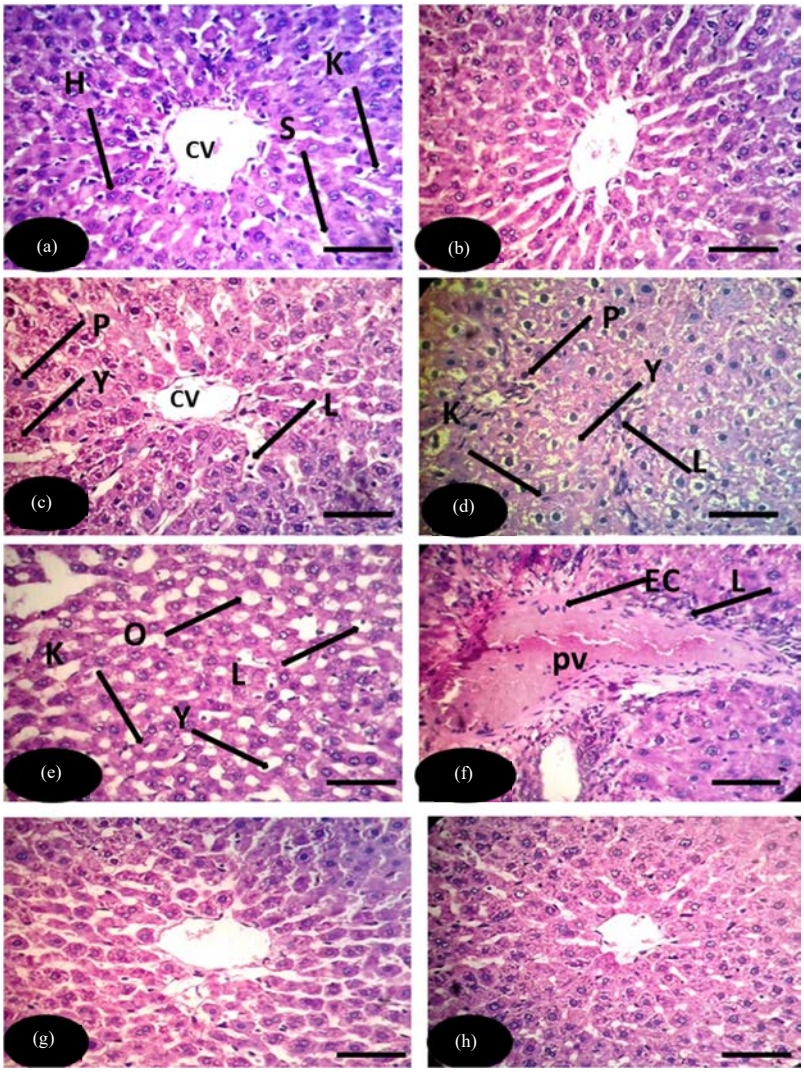

Fig. 4(a-h): Histopathological alterations induced by aflatoxin alone or combined with clay and nano clay treatment

(A) Section in rat liver of control showing normal appearance hepatic cells (H) radiating from a central vein (CV). Also, sinusoids (S) and kupffer cells (K) are noticed. (B): Section in the liver of rat treated with (5 g kg^{-1}) clay showing normal appearance of hepatocytes radiating from the central vein. (C): the liver of rat treated with (5 g kg^{-1}) nano clay showing some pyknotic nuclei(p) with infiltration of lymphocytes(L) and karyolysis of some nuclei (Y). (D): Section in the liver of rat treated with aflatoxin (1mg kg-1 diet) showing karyolysis of some nuclei, all nuclei in the section are pyknotic, increasing of kupffer cells, absence of sinusoids, obvious degeneration in the cytoplasm and aggregation of lymphocytes. (E): Another section in liver rats treated with 1 mg kg^{-1} AF showing karyolysis of some nuclei, increasing of kupffer cells, many lipid droplets (O) and infiltration of lymphocytes. (F): Also another section in liver rats treated with 1 mg kg^{-1} AF showing portal area with focal aggregation of lymphocyte infiltrations, dilatation of portal vein (pv) and swelling of their endothelial cells (EC), also, absence of sinusoids. (G): The liver of rat treated with 1mg kg^{-1} AF+clay showing normal appearance of central vein, normal hepatocyte and slightly infiltration of lymphocytes. (H): Section in the liver of rat treated with 1 mg kg^{-1} AF+nano clay showing normal appearance of a central vein; normal hepatocytes and sinusoids are observed

damage ($p<0.05$) as indicated by an increase in tail length (6.90 µm), tail DNA% (5.87%) and tail moment (40.50) as compared to the groups G_1, G_2 and G_3 (1.37 µm, 1%, 1.37, 1.48 µm, 1.22%, 1.81 and 1.54 µm, 1.35%, 2.10, respectively) Table 5 and Fig. 3a-c, respectively. This elevated DNA damage was reduced by treatments with clay (G_5, 3.35 µm, 2.90% and 9.72) and nano clay (G_6, 2.30 µm, 1.95% and 4.49) (Fig. 3e-f, respectively). However, this reduction remained significantly higher compare to groups (G_1-G_3). No significant difference was noticed compare to groups (G_1-G_3).

Effect of aflatoxin, clay and nano clay treatments on histological changes in rat liver: The treatment that was similar to the negative control (Fig. 4a) was clay (Fig. 4b) but the nano clay (Fig. 4c) showed some changed in the liver. Some nuclei were pyknotic and karyolysis with infiltration of

Table 5: Comet assay parameters obtained by image analysis in rat liver

Groups	Tailed (%)	Untailed (%)	Tails length (μm)	Tail DNA(%)	Tail moment
G1	2	98	1.37±0.09[d]	1.00	1.37
G2	2.5	97.5	1.48±0.07[d]	1.22	1.81
G3	3	97	1.54±0.08[d]	1.35	2.10
G4	25	75	6.90±0.32[a]	5.87	40.50
G5	13	87	3.35±0.16[b]	2.90	9.72
G6	6.5	93.5	2.30±0.10[c]	1.95	4.49

G_1 (control): G_2 (control+clay): G_3 (control+nano clay): G_4 (AF): G_5 (AF+clay); G_6 (AF+nano clay); Data are expressed as Mean±SEM (n = 7), different superscript letters in the same column of tail length showed significance difference at $p<0.05$

lymphocytes in them. The most histological effects related to the treated rat with AF were karyolysis (Y) and pyknotic (P) nuclei, increasing of kupffer cells (K), absence of Sinusoids (S), degeneration in the cytoplasm and aggregation and infiltration of Lymphocytes (L), lipid droplet (O), dilatation of portal vein and swelling of their Endothelial Cells (EC), also, in some section the absence of sinusoids are noticed (Fig. 4d-f). These changed were reduced in the treatment with clay reduced the changes induced by AF (Fig. 4g). Also, the treatment with nano clay reduced the changes induced by AF (Fig. 4h).

DISCUSSION

The administration of aflatoxin with diet increases the activity of liver enzymes (Table 3), these agree with previous studies[8,28-30]. Increasing activity of AST may indicate degenerative changes and hypofunction of the liver[31]. Also, the increased levels of ALT indicating hepatocellular damage or necrosis[32]. The enzyme activities back to normal conditions when the rats were treated with clay and nano clay with AF. Also, Eraslan *et al.*[33] said that using bentonite brought the liver enzyme activities back to normal conditions. Abdel-Wahhab *et al.*[29] mentioned that there was no evidence of a deleterious effect on liver enzymes when organo-modified nano-montmorillonite was used alone. It succeeded to inhibit the toxic effects of aflatoxin.

All studies on aflatoxin indicated a significant reduction in antioxidant activity[34-36]. Our results indicated the effective role of clay and nano clay with AF in enhancing the antioxidant activity and this agree with Abdel-Wahhab *et al.*[29] they reported that animals treated with AFB1 plus organo-modified nano-montmorillonite showed improvement in the antioxidant enzymes activities in serum. In gene expression study aflatoxin showed a significant upregulation of CYP3A6 (one of Cytochrome P450 genes) expression levels as compared with the negative control. These findings indicated that CYP3A6 may play an essential role in defence against toxic insults. Due to their response to diverse toxic insults, it may also be used as excellent markers for liver toxicity[37]. Aflatoxins are very commonly found carcinogenic and oxidative agents that induced oxidative stress in the body[38]. This is very obviously in antioxidants results (Table 4). This upregulation in gene expression of CYP3A6 is due to the cell exposure to aflatoxin to face oxidative stress. However, only limited information is available regarding rat CYP3A6 and its role in the bioactivation of AF has been seldom reported. On the other hand, in the treatments G5, G6 showed a significant reduction in CYP3A6 mRNA expression level. This indicated that the cell exposure to aflatoxin is decreased and so, the CYP3A6 gene expression down-regulated. The results agree with Jaynes *et al.*[39] they mentioned that clays added to animal diet were found to decrease the harmful effects of aflatoxins, because of aflatoxin adsorption to clays.

AF induced the gene expression of Heme oxygenase-1 (HO-1) and this disagrees with many reports that mention the downregulation of HO-1 levels in Af-treated group[40]. We can explain the high expression in another view belong to the function of HO-1 and bilirubin. Bilirubin is a specific signal of hepatic injury. Other authors have reported an increase in plasma/serum bilirubin as a result of aflatoxin treatment in rats[41]. HO-1 degrades heme in an identical stereospecific manner to biliverdin. In mammals, biliverdin is rapidly reduced by biliverdin reductase to bilirubin[42]. These agree with the high levels of HO-1 gene expression levels induced by AF. Our results showed that HO-1 significantly increase in AF treatment and decrease in the other treatments. The treatment AF with clay and nano clay reduced the high levels of HO-1 induction by aflatoxin and this expression was close to the negative control levels[43].

The results refer to a significant increase of TNFα (one of the proinflammatory cytokine) in rats consumed the AF contamination feed compared to the control groups. In agreement with the study[44] reported that broiler hens fed aflatoxin had higher serum TNFα levels, which could be related to an acute-phase response to inflammation generated by the toxic effects of AF. These changes may be associated with oxidative stress by AFB1, which was supported by previous studies[45,46]. The gene expression profile suggested that an AF increase TNFα and there was a restorative effect

when diet supplemented with clay and nano clay that seemed to counteract the immunosuppression of AF[47].

NF-κB was activated in AF-treated rats. Ma *et al.*[48] proposed that AFB1-induced elevation of inflammatory cytokines was attributed to up-regulation of the NF-κB signalling pathway. The present study demonstrated the relationship between TNF-α and NF-κB expression in the liver of AF-induced rats. Cumulative evidence suggested that inflammatory response could facilitate structural and functional liver injury[49]. The increase of inflammatory cytokines was an important factor for causing liver cancer[50]. The treatment G_5 and G_6 can downregulate NF-κB and reduce pro-inflammatory cytokines production[51]. The mechanisms of action of this downregulation because of the clay and nano clay prevent a lot of aflatoxin from arriving at the cell and induce damage.

We found a markedly decrease in Glucose transporter type-2 (GLUT2) mRNA level in liver cells was triggered by AF. The treatment G_5 or G_6 reduced the effect of aflatoxin. They upregulated the gene expression, indicating that clay and nano clay could enhance GLUT2 gene expression in keeping regular nutrient transport and absorption in the intestines by reducing the amount of AF. There are few reports on the effect of aflatoxin and GLUT-2 and some disagree with the results in this study. Other mycotoxins (Fumonisin B_1) agree with our result where it down-regulates the expression of GLUT-2[52]. In some reports, AF didn't affect the sugar transporters (GLUT2)[34]. On the contrary, the other reports indicate the upregulation of GLUT2 with treatment by AF[53]. Mycotoxicosis decreased the expression of GLUT-2[54].

Treatment G_5 and G_6 resulted in a significant improvement in reducing DNA fragmentation induced by AF. Our results agree with Bakheet *et al.*[55] they mention that the incidence of DNA strand breaks observed in AFB1-treated rats was significantly higher than that of G_1. Tigran *et al.*[56] suggest that prolonged exposure to mycotoxins combination through food consumption can induce DNA damage contributing to the harmful effects *in vivo*. Our results agree with Vipin *et al.*[57] In comparison to vehicle control cells, the AFB1 treatment for 24 hrs enhanced the length of DNA in the comet tail.

The histological results in this study revealed that the liver of the animals in the AFs-treated group showed severe histological changes typical to those reported in the literature[58-60,37]. The addition of clay and nano to the aflatoxin-contaminated diet alleviated the negative effects of aflatoxin[9]. These results support the hypothesis that clay or nano clay bind the AF in the gastrointestinal tract of the animals and the clay-mycotoxins complex is stable and does not affect by the different metabolizing enzymes. The addition of nano clay as a feed additive has become a subject of increased interest in animal nutrition. Dietary supplementation with a nano-clay adsorbent for broilers also holds promise in improving growth performance and reducing the toxic effects of aflatoxin contamination[61]. Our results indicated that there was a potential for reducing the toxicity of aflatoxin by using a low expensive and safe natural material and the nano form of it, so we can be recommended by adding this material to the feed of poultry and animal because reduced the accumulation of aflatoxin in the tissues and so reducing this mycotoxin to reach to human.

CONCLUSION

Our work suggests that clay is a high-affinity adsorbent for AF in rats and that the use of nano clay may offer an amoral approach to ameliorative management of aflatoxicosis in animals. This is the first time that it has been shown that clay and nano clay reduce NF-κB activation in AF-treated rats.

SIGNIFICANCE STATEMENT

This study discovers the possible synergistic effect of clay and nano clay in eliminating the side effects of aflatoxin. This study will help the researchers for the first time in linking the different effects of aflatoxin at the genetic level as well as the biochemical and histological levels with the use of new materials in this field at such combined levels of study.

ACKNOWLEDGMENT

The authors appreciate the support of Dr. Yousef Sultan Associate Professor in Food Toxicology and Contaminants Department, National Research Center, Dokki, Cairo, Egypt for providing the fungal strain.

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