

Biochemical studies on the protective effects of *Egyptian Montmorillonite clay* and Activated Carbon against health hazards resulting from the exposure to Deoxynivalenol mycotoxin in food

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Abstract

The current study aimed to use the *Egyptian Montmorillonite* clay (EM clay) and Activated Carbon (AC) derived from dates stones as a protective agent against Deoxynivalenol toxicity. Forty eight female Sprague-Dawley rats were divided into six groups including the control group, the group fed basal diet plus EM clay (0.5%), the group fed basal diet plus AC (0.5) % and other three groups fed DON- contaminant diet (25 mg / kg diet) alone or plus EM clay (0.5 %) or plus AC (0.5%). At the end of the experimental period, blood samples were collected for serum biochemical analyses. Animals were scarified and dissected samples of liver and kidney were collect for histopathology and determination of antioxidant enzyme activities and lipid peroxidation in liver. The results indicated that rats fed DON-contaminated diet showed a significant increase in the activities of ALT, AST, ATP and levels of uric acid, createnin, total lipids, triglycerides and lipid peroxidation and also showed significant decrease in Total Antioxidant Capacity (TAC), Nitric oxide activities and significant decreases in GSH level. Histological examination of the liver and kidney tissues showed severe histopathological and histochemical changes. Animals fed DON-contaminated diet plus EM clay or AC showed a significant elimination of the harmful effects of DON in all biochemical, antioxidant parameters and the histological picture of liver and kidney.

Keywords: Deoxynivalenol, Biochemical Parameters, Activated Carbon, *Egyptian Montmorillonite clay*

1. Introduction

Mycotoxins are secondary metabolites produced by fungi, mainly by species from the genus *Aspergillus*, *Fusarium* and *Penicillium*. The toxicological syndromes caused by ingestion of such toxins range from acute mortality, to slow growth and reduced reproductive efficiency [65]. Consumption of fungal toxins may also result in impaired immunity and decreased resistance to infectious diseases [29,44,45]. The global occurrence of mycotoxins is considered an important risk factor for both human and animal health [22].

Deoxynivalenol (DON), also known as vomitoxin, is a type B trichothecene, which is found naturally worldwide in a variety of animal feeds and human foodstuffs, particularly in cereal grains, such as wheat, barley, maize, and flour [47]. Furthermore, this toxin is resistant to milling, processing and heating, and, therefore, readily enters human and animal food chains [65].

DON exhibits toxic effects in humans as well as in all animal species so far investigated [60]. The initial adverse effect observed after DON exposure is reduced feed intake. Ingestion of DON has also been associated with gastroenteritis as reflected by nausea, emesis, diarrhea, anorexia and gastrointestinal hemorrhaging [49].

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At the cellular level, DON binds to ribosomes and inhibits protein synthesis [20]. Actively dividing cells, such as those of the immune system, are a primary target for DON [50,51]. Ingestion of DON-contaminated feed modulates the immune response in different species including rodents, human, avian and swine [60]. Depending on dose and frequency of exposure, DON can be either immunosuppressive or immunostimulatory. In mice, acute high-dose DON exposure causes rapid onset of leukocyte apoptosis [50].

Clays are widely applied in many fields of technology and science since the wide usefulness of clays resulted from their high specific surface area, high chemical and mechanical stability and a variety of surface and structural properties [32,38]. Jiang and Zeng (2003) [32] reported that chemisorbent materials play an important role in the removal of heavy metals, natural organic matter and synthetic organic compounds. Several reports indicated that, clays have unique properties, such as their high specific surface areas associated with their small particle size, low cost, and ubiquitous occurrence in most soil and sediment environments [13].

The adsorption on activated carbon is one of the more popular methods for the removal of pollutants from aqueous solutions [9]. On the other hand, agricultural by-products and wastes present highly recommendable sources because they are readily available, low-cost, regularly produced and renewable feed stocks. Moreover, the use of clays minerals as adsorbent agents to remove chemical contaminants had been increasingly paid attention because they are cheaper than other materials such as activated carbon.

The aim of the current study was to evaluate the protective effects of EM clay and AC against DON-induced oxidative stress in rats.

2. Materials and methods

Chemicals and adsorbent: TDON standards and all chemicals used in the current study were of the highest purity commercially available and were purchased from Sigma Chemical Co. (St. Luis, Mo, USA). The Egyptian Montmormolinite (EM) clay used in this study was provided by the Materials Science Department, National Research Center, Cairo, Egypt.

Physico-chemical characterization of The Egyptian Montmormolinite clay and Activated Carbon: The results of the chemical analysis of EM clay

revealed that was composed of 52.61% SiO₂, 10.11% Al₂O₃, 5.10% Fe₂O₃, 0.44% TiO₂, 1.05% MgO, 5.75% CaO, 2.22% Na₂O, 2.30% K₂O, 20.19% FL, and 0.19% SO₃ based on the dry weight. The loss of weight after heating at 1000°C was 25.03%. The cation exchanges capacity (CEC) was 130 meq/100 g and the specific surface area was 555.0 m²/g. The main infrared absorption bands for purified bentonite were located at 3450, 997, 814 and 680 cm⁻¹, and were attributed to water stretching vibration, symmetric deformation of Si–O–Si and Si–O–Al groups, Al–OH and Al–O deformation. The X-ray patterns for the original and purified bentonite indicated high purity and crystallinity without impurities of SiO₂ (quartz and hematite) and carbonate. Characterization of AC previously described in our study [64].

Kits: Kits of aspartate transaminase (AST), alanine transaminase (ALT), Total Antioxidant Capacity (TAC), Triglycerides (TG), Creatinin, Alkaline Phosphatase (ALP), Uric Acid, Total lipids (TL), Glutathione Reduced (GSH), Nitric oxide (NO) and Lipid Peroxidation were obtained from Biodiagnostic Co., El-Dokki- Giza.

In vivo study: Preparation of DON-contaminated diet: The DON was produced by the fermentation of corn by *Fusarium graminearum* ITEM 126 as described by Kamimura et al., 1981 [33]. The fermented corn was autoclaved; ground to a powder and the DON content was measured by HPLC [73]. The corn powder was incorporated into the basal diet to provide the desired level of 25 mg/kg diet. The diet containing DON was analysed and the presence of parent AFs was confirmed by HPLC.

Animals and treatment: Fourty eight sexually mature female Sprague–Dawley rats (12-week-old) weighing 100–130g (provided by the Animal House Colony, National Research Center, Dokki, Cairo, Egypt) were maintained on the standard laboratory diet (protein: 16.0%; fat: 3.6%; fiber: 4.1%, and metabolic energy: 0.012 MJ) and water ad libitum at the Animal House Laboratory, National Research Center, Dokki, Cairo, Egypt. After an acclimation period of 1week, the animals were distributed into four groups (10 rats/group) and housed in stainless steel cages in a temperature-controlled (23±1 °C) and artificially illuminated (12 h dark/light cycle) room free from any source of chemical contamination. All animals received humane care in compliance with the guidelines of the Animal Care and Use Committee of National Research Center,

Egypt. Animals within different treatment group Animals within different treatment groups were treated daily for eight weeks as follows: group 1, untreated control; group 2, treated with EM clay (0.5g/kg feed) mixed with standard diet; group 3, treated with Activated carbon (0.5 g/kg feed) mixed with a standard diet; group 4, (25mg DON /kg feed); group 5, treated with (0.5g clay /kg feed +25mg DON /kg feed) mixed with a standard diet. At the end of the treatment period, blood samples were collected from the retro-orbital venous plexus and the sera were separated and stored at -20 °C until analysis.

Biochemical analyses: Serum ALT and AST activities were determined according to the method recommended by Reitman and Frankel, (1957) [59], ALP [61], TriG [69] Cho [14], TL [72]; creatinine [10]; uric acid [28]; TAC [35] and NO [42].

After blood samples were collected, all animals were killed and sample of liver and kidney tissues of each animal was dissected, weighed and homogenized in phosphate buffer (pH 7.4) to give 20% w/v homogenate [37]. This homogenate was centrifuged at 1700 rpm and 4 °C for 10 min and the supernatant was stored at -70 °C until analysis. Lipid peroxidation (LP) was estimated by measuring the formed malondialdehyde (MDA) using thiobarbituric acid reactive substances method according to the spectrophotometric method of Buege and Aust (1978) [12] and Ruiz-Larrea et al. (1994) [62].

The level of lipid peroxide was expressed as nmol MDA per gram tissue. Other samples of the liver and kidney were excised and fixed in natural formalin and were hydrated in ascending grades of ethanol, cleared in xylene and embedded in paraffin. Sections (5 mm thick) were cut and stained with hematoxylin and eosin (H & E) for the histological examination [18].

Statistical analysis: All data were statistically analyzed using the General Linear Models Procedure of the Statistical Analysis System [63]. The significance of the differences among treatment groups was determined by Waller–Duncan k-ratio [70]. All statements of significance were based on probability of $P \leq 0.05$.

3. Results

Effect of DON on feed intake and body weight of rats: The results of the current study revealed that no animal mortality was observed in any of the treatment groups except only one rat was died in the group fed DON-contaminated diet (25mg/kg diet) for 8 weeks. Moreover this group showed some symptoms such as vomiting, weakness and low activity. The acute toxicity of DON firstly appeared as a significant decrease in feed intake. Animals fed DON-contaminated diet alone showed a significant decrease in feed intake compared to the control group. But Animals fed DON-contaminated diet plus AC or/and EM clay not had significant decrease in feed intake. Furthermore, Animals fed basal diet plus AC or/and EM clay showed significant improvement in feed intake comparable to the toxin group (Table 1, Fig 2). Our current results in (Table 1, Fig 1) showed that Animals fed DON-contaminated diet (25mg/kg diet) alone for 8 weeks showed a significant decrease in body weight compared to control groups. Results in the same table and Figure indicated that animal in groups fed basal feed plus AC and/or EM clay (0.5%) had no significant differences noticed in body weight compared to control group. Animals fed DON-contaminated diet plus AC and EM clay showed a significant improvement in body weight compared to group fed DON-contaminated diet alone.

Table 1. Effect EM clay and AC on body weight gain and feed intake of rats fed DON contaminated diet for eight weeks (means \pm SE)

Parameter	Daily Feed intake (g)	Body weight (g)
Control	20.52 \pm 0.63 ^a	174.72 \pm 6.24 ^a
EM clay	19.25 \pm 0.46 ^a	170.98 \pm 4.85 ^a
AC	19.81 \pm 0.46 ^a	171.98 \pm 0.85 ^a
DON	13.68 \pm 0.37 ^b	140.5 \pm 1.05 ^b
EM clay +DON	19.13 \pm 0.57 ^a	170.41 \pm 1.99 ^a
AC + DON	19.72 \pm 0.48 ^a	170.61 \pm 0.81 ^a

Within each column, means superscript with different letters are significantly difference ($P \leq 0.05$)

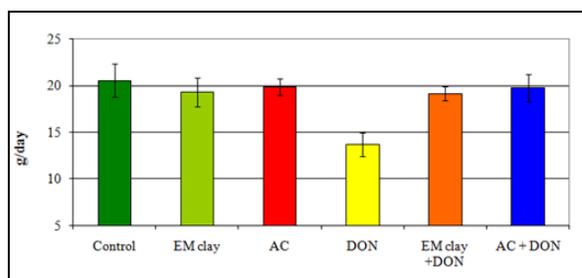


Table 1. Effect of EM clay and AC on daily feed intake in rats DON- contaminated diet for 8 weeks

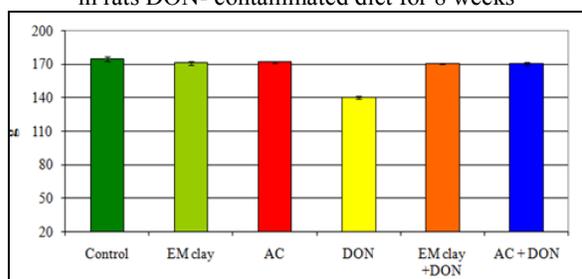


Table 2. Effect of EM clay and AC on body weight gain in rats DON- contaminated diet for 8 weeks

The biochemical study: The results of the present study revealed that DON induced severe toxicological effects on serum biochemical parameters tested. Results of serum biochemical parameters are depicted in (Table2). Animals fed DON-contaminated diet (25mg/kg diet) alone for eight weeks showed a significant increase in serum ALT, AST and ALP, TriG, NO, Createnin, and Uric acid accompanied with a significant decrease in GSH and Total Antioxidant Capacity in liver tissue (TAC) compared to control group. Animals fed basal diet plus AC and/or EM clay (0.5%) for 8 weeks had no significant differences in serum TAC, TriG, NO, createnin and uric acid compared to control group. The combined treatment of DON plus the EM clay or/and DON plus AC succeeded to induce a significant improvement in all the tested parameters toward the control level.

This decrease was pronounced in the group fed DON plus AC. The current results indicated that animals fed DON-contaminated diet alone showed a significant increase in MDA in liver and kidney compared to the control group.

Animals treated with the EM clay and AC alone showed a significant decrease in MDA in liver and kidney compared to the control animals. The combined treatment succeeded to induce a significant improvement in MDA in liver and kidney. This improvement was striking in the group fed DON-contaminated diet plus AC (Table 3).

The histological examination of the liver in the control group showed normal histological structure of the liver lobule and the central vein and hepatocytes separated with blood sinusoids (Fig. 3a). The liver of animals treated with the EM clay and AC showed normal hepatocytes and portal tract (Fig. 3b, 3c). The liver section of rat fed DON-contaminated diet showed enlarged portal area and dilated thick wall portal vein and also appeared accumulation of cellular infiltration and fibrous tissues around proliferated bile ducts. Thickening of the interlobular connective tissue and massive inflammatory infiltration and congested and dilated veins in the portal areas also the hepatocytes that surrounded the portal areas showed necrosis associated with inflammatory infiltration. The hepatocytes showed vacuolar degeneration and nuclear pleomorphism (Fig. 3d, 3e). The liver of animals fed DON-contaminated diet plus EM clay or/and AC showed a significant improvements in liver tissues and normal hepatocytes (Fig. 3g).

Microscopic examination of kidney section of control rats showed the normal structure of renal tissue in convoluted tubules and the Bowman capsule (Fig. 4a). Also animal fed basal diet plus protective EM clay and protective AC showed the nearly normal structural of renal tissue and normal convoluted tubules and the Bowman capsule (Fig4, b, c). The kidney of rats fed DON-contaminant diet alone showed some distal tubules have fatty degeneration and eosinophilic cytoplasm as well as pyknotic nuclei. Interstitial edema and inflammation also present (Fig 4 a, b). Kidney tissue of rats fed DON-contaminated diet plus EM clay showed nearly normal tubules and glomeruli, also kidney of the animal in group six that fed DON-contaminant plus AC showed most of proximal or distal tubules and glomeruli in normal structure (Fig 4 e, f).

Table 2. Effect of EM clay and AC on different biochemical parameters in rats of EM clay and AC on different biochemical parameters in rats fed DON-contaminated diet (25 mg/kg diet) for eight weeks.

Parameters	Groups					
	Control	EM clay	AC	DON	DON +EM clay	DON + AC
ALT (U/L)	28.59±1.78 ^a	29.69±2.30 ^a	29.54±3.46 ^a	69.7±2.07 ^b	32.19±2.50 ^a	29.81±0.19 ^a
AST (U/L)	32.85 ± 2.83 ^a	33.97 ± 2.46 ^a	33.29 ± 2.17 ^a	87.11 ± 1.12 ^b	35.44 ± 2.20 ^a	33.65 ± 1.45 ^a
ALP (U/L)	29.03±4.51 ^a	27.93±2.80 ^a	30.76±6.12 ^a	76.1±11.77 ^d	36.92±3.60 ^b	34.12±2.52 ^c
TRiG (U/L)	89.90±4.37 ^a	92.42±1.79 ^a	87.95±6.02 ^a	131.47±9.08 ^b	87.31±7.29 ^a	87.66±4.82 ^a
Cho (mg/dl)	125.14±2.63 ^a	127.84±3.40 ^a	126.81±4.20 ^a	2.18.48±2.81 ^b	140.35±3.81 ^a	136.36±2.62 ^a
TL (mg/L)	2.99±0.31 ^a	3.26±0.36 ^b	3.6±0.42 ^b	5.86±0.21 ^c	3.67±0.21 ^b	3.16±0.38 ^a
Createnin(mg/dl)	1.43±0.31 ^a	1.52±0.22 ^{ab}	1.82±0.11 ^b	1.48±0.17 ^a	1.53±0.20 ^{ab}	1.48±0.17 ^a
Uric acid(mg/dl)	11.07±1.17 ^a	12.88±0.90 ^{ab}	11.73±1.79 ^a	14.44±2.30 ^b	12.08±2.5 ^{ab}	11.48±2.04 ^a
TAC (mmol/g liver tissue)	1.43±0.16 ^a	1.47±0.24 ^a	1.47±0.19 ^a	1.14±0.8 ^b	1.46±0.35 ^a	1.47±0.25 ^a
NO (mmol/L)	17.91±2.06 ^a	19.38±1.69 ^a	18.51±2.03 ^a	37.62±1.53 ^b	18.16±2.63 ^a	18.23±2.21 ^a
GSH (mmol/L)	14.53±1.13 ^a	14.33±1.45 ^a	16.53±1.05 ^b	12.2±1.07 ^c	17.73±1.35 ^b	16.2±1.96 ^a

Within each row, means superscript with different letters are significantly different ($P \geq 0.05$). (ALT, AST: Transaminase; ALP: alkaline phosphatase; Cho: cholesterol; TriG: triglycerides; TL: total lipids; TAC: total antioxidant capacity, NO: nitric oxide, GSH: Glutathione reduced)

Table 3. Effect of EM clay and AC on lipid peroxidation in liver and kidney of rats fed DON-contaminated diet (25 mg/kg diet)

Croups	MDA (nmol/mg protein)	
	Liver	Kidney
Control	62.84±1.46 ^a	6.74±1.5 ^a
EM clay	64.15±1.39 ^a	6.18±1.02 ^a
AC	61.43±1.51 ^a	6.13±0.33 ^a
DON	98.66±1.51 ^b	10.74±1.24 ^b
DON+ EM clay	66.31±1.55 ^c	5.07±1.51 ^a
DON + AC	63.18±1.49 ^a	6.11±1.42 ^a

Within each column, means superscript with different letters are significantly different ($P \geq 0.05$). MDA: malondialdehyde

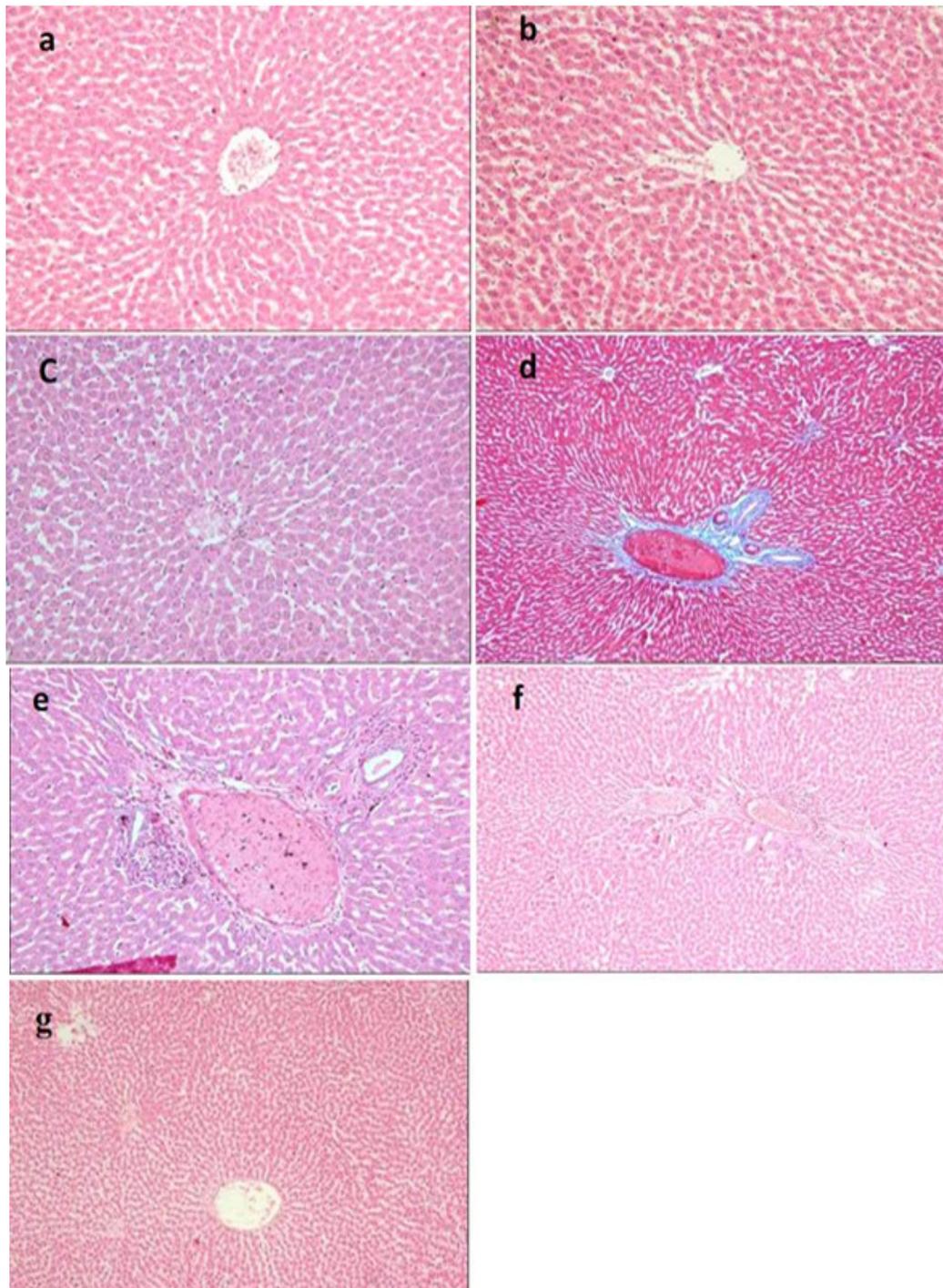


Figure 3. A photomicrograph in liver sections of (a) control rats showing the normal histological structure of liver lobule, central vein and hepatocytes separated with blood sinusoids, (b) rat treated with EM clay showing nearly normal hepatocytes and portal tract, (c) rat treated with AC showing nearly normal hepatocytes and portal tract, (d,e) rat fed DON- contaminated diet showing marked increased in the collagen fibers around the portal tracts and a minimum around the central vein, thickening portal tract with cellular debris and periportal fibrosis. The hepatocytes showing vacuolar degeneration and nuclear pleomorphism, (f) rat fed DON- contaminated diet plus EM clay showing hepatocytes with less vacuolar degeneration and nuclear pleomorphism, (g) rat fed DON-contaminated diet plus AC showing significant improvements in liver tissues and normal hepatocytes

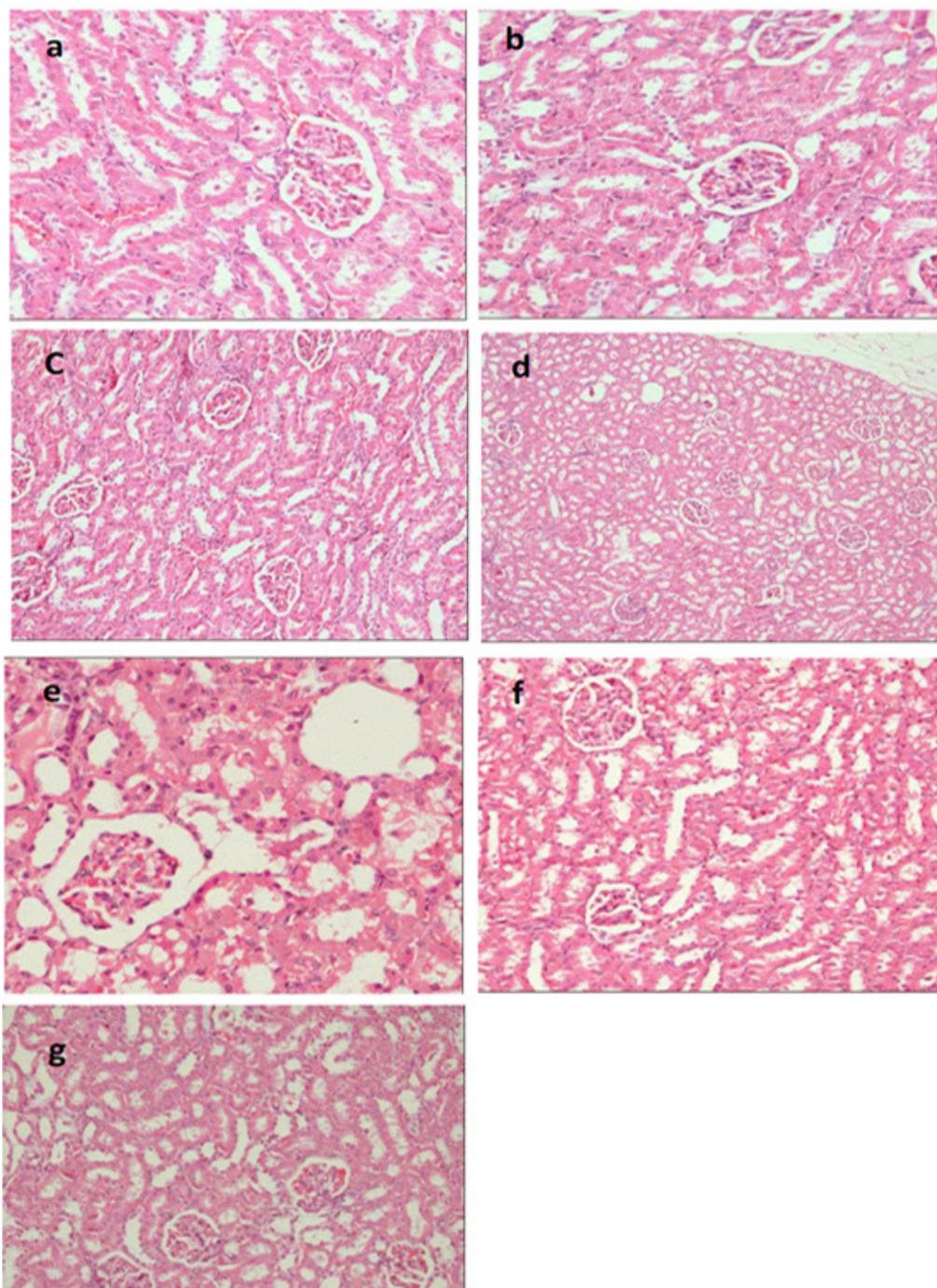


Figure 4. A Microscopic examination of kidney sections of (a) control rats showed the normal structure of renal tissue in convoluted tubules and the Bowman capsule; (b,c) animal fed basal diet plus protective EM clay and protective AC showed the nearly normal structural of renal tissue and normal convoluted tubules and the Bowman capsule. (d,e) The kidney of rats fed DON-contaminant diet alone showed some distal tubules have fatty degeneration and eosinophilic cytoplasm as well as pyknotic nuclei. Interstitial edema and inflammation also present; (f) Kidney tissue of rats fed DON-contaminated diet plus EM clay showed nearly normal tubules and glomeruli; (g) kidney of the animal in group six that fed DON contaminant plus AC showed most of proximal or distal tubules and glomeruli in normal structure

4. Discussion

The sensitivity of laboratory animals to DON relative to poultry and ruminants can be explained, in part, by the rapid and deficient absorption, extensive systemic distribution and poor metabolism of DON in pigs [25,54,55]. In the same concern, Guan et al. (2009) [74] recently attributed the variability in sensitivity to DON among several species of rats to a differential ability of the intestinal microbes to transform DON to DOM-1.

In the current study we evaluated the ability of EM clay and AC to protect the laboratory animals from toxic effect of DON. The selective dose of DON and adsorbent agent were based on our in vitro study and literature [23,64]. Our results indicated that ingestion of DON mycotoxin resulted in vomiting and a significant decrease in food intake and consequently the body weight gain was also reduced. Similar decrease in food consumption and body weight was reported in rats fed DON-contaminated diet by Forsell, et al 1987, Raymond et al., 2005; Pestka., 2010; Amuzie et al., 2011 [7,23,48,58]. The reduced feed intake may indicate protein catabolism thereby contributing to the observed kidney injury and causing impaired glomerular filtration [68]. On the other hand, the decrease in body weight in the animals treated with the mycotoxin alone may be due to the effects of these mycotoxins on the balance between orexigenic and anorexigenic circuits that regulate the homeostatic loop of body weight regulation, leading to cachexia [57]. In this regards, Amouzie et al. (2009) [6] stated that DON suppresses growth in experimental animals by reducing growth hormone (GH) signaling through mechanisms mediated by insulin-like growth factor 1(IGFI) and insulin-like growth factor acid-labile substance (IGFALS). DON causes the ribotoxic stress response in rats and, as a result, there is activation of MAPKs and up-regulation of proinflammatory cytokines. Among the latter are interleukin-6 (IL-6) and tumor necrosis factor- α (TNF- α) both of which have been shown to exert a negative influence on weight gain when over-expressed [16,56]. In addition, proinflammatory cytokine induction up-regulates the expression of suppressors of cytokine signaling (SOCS). SOCS proteins negatively modulate cytokine signaling and have been shown to inhibit growth hormone (GH)-induced gene expression in liver [75]. Therefore, SOCS could be up-regulated and subsequently have a negative impact growth in DON-exposed animals. Amouzie et al. (2009) [6]

recently demonstrated that DON did increase expression of SOCS mRNAs, including those for SOCS₁, SOCS₂, SOCS₃ and CIS (cytokine inducible SH₂ domain protein) in a dose-dependent and transient (although SOCS₃ remained elevated longer than the other SOCS proteins) manner. SOCS up-regulation occurred together with or shortly after increases in TNF- α and IL-6mRNA and protein expression were observed. They also established in their earlier study, that hepatic IGFALS mRNA expression was suppressed in mice given DON, thus providing an important clue suggesting that there is a link between DON-induced up-regulation of pro-inflammatory cytokine signaling and suppressed of growth through inhibition of IGF1 and IGFALS. The DON-related decrease in feed intake observed in this study has been extensively documented in terrestrial species and is believed to be closely related to the effect of DON on brain neurotransmitter concentrations [53,66]. Oral dosing or consumption of DON has been correlated with significantly elevated brain concentrations of serotonin (5-hydroxytryptamine) in rats and pigs [67]. Although the response of brain monoamines to DON has not been determined in aquatic species, serotonin has previously been implicated as an appetite suppressor in fish and laboratory animals [17,30,39]. The effect of DON on regional brain neurochemistry may have contributed to the reduction in feed intake of rats exposed to the increasing dietary concentrations of DON in this study.

DON could induce DNA fragmentation in chicken spleen leukocytes using comet assay [24]. Also comet assay showed that DON was capable of inducing single-strand breaks (SSBs) in human Caco-2 cells and the DNA damage increased in a dose-dependent manner [11]. DON was reported to induce lipid peroxidation as measured by malondialdehyde (MDA) production in Caco-2 cells and liver cell [36] and increased formation of thiobarbituric acid-reactive substances (TBARS) in a dose response manner suggests that it is capable of yielding radical species that could cause DNA damage. With respect to its carcinogenicity, a high incidence of lung carcinoma and dysplasia of glandular stomach were diagnosed in mice orally exposed to the toxin over 24 weeks [31].

In the present study, DON was used to induce toxicity in rats. Our data revealed that DON induce a significantly increase in ALT, AST and ALP activities, uric acid, creatinin, triglycerides and total

antioxidant capacity. The activity of ALT and AST are sensitive indicators of acute hepatic necrosis [34]. Consequently, these results may indicate degeneration change of liver and kidneys [21]. The increase level in uric acid and creatinin reported herein may indicate. The protein catabolism and/or kidney dysfunction [4]. These results clearly showed that DON had harmful and stressful influences on the hepatic and renal tissue and consistent with those reported in the literature [47]. On the other hand, the significant increase in the triglycerides in the group fed DON-contaminant diet alone are coincided with those reported previously in AFs-ingested animals [19]. This elevation in serum triglycerides is probably associated with biliary obstruction and acute hepatic injury [19]. Similar to our observations Pestaka et al, (2006) [51] stated that DON can diminished liver activity in rats and supported the hypothesis that rodent liver is one of a targets tissue of DON toxicity [51].

Lipid Peroxidation (LPO) is one of the main manifestations of oxidative damage and has been found to play an important role in the toxicity and carcinogenesis of many carcinogens [57]. In the same regard, Kouadio et al., (2005) [36] reported DON to induce lipid peroxidation as measured by malondialdehyde (MDA) production in liver cells and Caco-2 cells and increased formation of thiobarbituric acid-reactive substances (TBARS) which might be suggests capable of yielding radical species that could cause DNA damage. Another mechanism by DON may induce or promote hepatic toxicity is through the increase of hepatic oxidative stress [27]. This oxidative stress results in enhanced lipid peroxidation (LOP) leading to cell membrane damage, DNA damage, cellular apoptosis or necrosis and tumorigenesis [36]. The significant increase in LOP in rats fed DON-contaminant diet alone indicator to DON may cause oxidative stress in rats liver.

Our data showed that DON induced a significant increase in TAC in rats fed DON-contaminated diet alone. The current results coincided with those of Abdel-Wahhab. (2010) [2] who stated that aflatoxin as mycotoxin reduced level of TAC in rats fed Afls-contaminant diet (2.5 mg/kg diet). In a previous work, Abdel-Wahhab, (2010) [2] stated that TAC includes enzymes such as superoxide dismutase (SOD), catalase, and glutathione peroxidase (GPX) and macromolecules such as albumin, ceruloplasmin, and ferritin. TAC may provide more relevant biological information compared with that

obtained by measurement of its individual components, because it considers the cumulative effect of all antioxidants present in plasma and body fluids. GPX and SOD are considered enzymatic free radical scavengers in cells [26].

In the present study, the decrease in TAC in rats fed DON-contaminated diet alone might indirectly lead to an increase in oxidative DNA damage [15]. On the other hand, SOD plays an important role in the elimination of reactive oxygen species (ROS) derived from the peroxidative process in liver tissues [46]. SOD removes superoxide by converting it to H₂O₂, which can be rapidly converted to water by catalase [2]. Furthermore, the reduced level of TAC may be explained by the association of GPX with DON or its metabolites [2]. These results coincided with those of Popovic, et al, (2004) [52] who stated that DON and ZEA can induce decrease in TAC level in rats and other rodent. In the same concern, several studies on the mechanisms of DON-induced liver injury have demonstrated that glutathione plays an important role in the detoxification of the reactive and toxic metabolites of DON, and the liver necrosis begins when the glutathione stores are almost exhausted [2,41] which may explain the decrease in TAC.

According to Moon et al. (2000) [43], Nitric oxide (NO) is produced by macrophages and it plays an important role in tumor conditions. The generation of NO by the inducible nitric oxide synthases (iNOS) plays a key role in the cytokine-mediated cell destruction [8]. In the current study, the ingestion of DON caused significant increase of NO level, suggesting that DON preferentially affects macrophage functions. It is well known that the enzymatic antioxidant play a substantial role in protecting organisms against oxidative stress. In the absence of these enzymatic antioxidants, hydroxyl radicals, the causative agent of LOP, will attack polyunsaturated fatty acids to produce lipid peroxides in the presence of transition metals such as iron which coupled with a redox system and oxygen under appropriate conditions. The current results coincided with those of Zhang et al. (2009) [71] who stated that DON may increase hepatic LOP by lowering cellular antioxidant defenses.

The histological results reported in the current study confirmed the biochemical results and indicated that DON induced severe histological change in liver and Kidney. The histological changes of liver induced by DON have been documented as a result

of treatment with aflatoxin previously by Mayura et al., 1998, Aabdel-Wahhab et al., 1998, 2010 [2,3,40]. The present results indicated that EM clay and AC succeeded to prevent the histological changes which clearly indicate that EM clay tightly bind DON in the gastrointestinal tract and reduce the bioavailability of the toxin and reduce absorption of toxin to blood stream. These results are supported by the result of in vitro study as well as findings of Aly et al. (2004) [5] who reported that EM clay succeeded to absorb more than 92.2% of the available of aflatoxin B1 in malt extract. In the present study a protective effect of EM clay and AC against the toxic hazard of DON was observed. The treatment with EM clay and AC in the presence of DON led to the reduction of the elevated levels of the biochemical parameter (ALT, AST, ALP, creatinin, uric acid, TL, TAC, TriG, NO, GSH and lipids peroxides) and normalized the histological picture of the investigated organs. Present results coincide with those of Abbes et al. (2008) [1] who stated that EM clay was found to be safe and successful in the prevention of aflatoxin toxicity.

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