Toxin Reviews

ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/itxr20

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To cite this article: Sheraz Ahmed Bhatti, Muhammad Zargham Khan, Muhammad Kashif Saleemi, Zahoor Ul Hassan & Ahrar Khan (2022) Ameliorative role of dietary activated carbon against ochratoxin-A induced oxidative damage, suppressed performance and toxicological effects, Toxin Reviews, 41:1, 108-118, DOI: 10.1080/15569543.2020.1848870

To link to this article: https://doi.org/10.1080/15569543.2020.1848870

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TOXIN REVIEWS
2022, VOL. 41, NO. 1, 108–118
https://doi.org/10.1080/15569543.2020.1848870

RESEARCH ARTICLE

Ameliorative role of dietary activated carbon against ochratoxin-A induced oxidative damage, suppressed performance and toxicological effects

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ABSTRACT

The present study reports that dietary addition of 2.5, 5.0, 10.0 g/kg activated charcoal (AC) in broiler feed contaminated with 0.15, 0.3 and 1.0 mg/kg ochratoxin A (OTA) showed a partial reduction in intensity of mycotoxins induced toxic effects; particularly against those induced at lower doses of toxin (0.15 and 0.3 mg/kg). The protection by AC was absent against 1.0 mg/kg OTA-mediated toxicities. Moreover, AC at 10 g/kg feed also imparted toxicological effects in broiler chicken. It can be concluded that AC possess limited adsorptive potential against OTA and increasing the dose of AC may be detrimental for animal health.

ARTICLE HISTORY
Received 11 April 2020
Revised 3 November 2020
Accepted 7 November 2020

KEYWORDS
Ochratoxin A; broiler chicken; toxicopathology; oxidative stress; activated carbon

Introduction

Ochratoxin A (OTA), a low molecular weight secondary metabolite of certain toxigenic strains of *Aspergillus* and *Penicillium* species, is an inevitable natural contaminant of feed and foodstuff throughout the world. Ambient environment conditions (temperature, pH and moisture) favor these toxigenic filamentous fungi to proliferate and results in accumulation of mycotoxins to the level deleterious to animal and human health (Milani 2013). OTA, among three sub-groups of ochratoxins (A, B and C), is the second most important mycotoxin after aflatoxin B1 due to its related toxic effects and the economic losses (Indresh & Umakantha 2013). Based on exhibited carcinogenicity of OTA in animal trials, International Agency for Research on Cancer (IARC) has classified it "possibly carcinogenic to humans" (group 2B) (IARC 1993). *In-vivo* experimental studies with different animal species have also confirmed the OTA induced nephrotoxicity, hepatotoxicity, immunosuppression, teratogenicity and mutagenicity (Qi *et al*. 2015, O'Brien & Dietrich, 2005). Oxidative stress resulting from OTA induced lipid peroxidation negatively influences the animal and human health by affecting the essential structural cells in the body even at a concentration below the safety threshold (Surai & Fisinin 2015, Periasamy *et al*. 2016). A particular danger is the residual accumulation of OTA in the edible tissues; poses a potential threat to public health due to carry-over into human food chain.

In avian species, feeding OTA contaminated diet result in significant health problems including the growth impairment, poor feed conversion ratio and marked degenerative change in the vital organs, like kidneys and liver (Khatoon *et al*. 2016). In the broiler chickens fed 200 µg OTA/kg for 3 and 4 weeks of age, an enlargement of liver and kidneys was observed whereas the microscopic lesion sore was highest for the kidneys followed by the liver (Santin *et al*. 2002). Severely decreased body weight of broiler chickens was observed after feeding 0.4 and 0.8 mg OTA/kg feed for five weeks of age (Elaroussi *et al*. 2006). In the chicks exposed to 0.5 mg OTA/kg feed for 6 and 10 weeks of age, severe macroscopic, histological, hematological and serum biochemical alterations (Stoev *et al*. 2002) indicated that the growth performance of the broilers birds is positively associated with OTA concentration in the feed and the duration of exposure.

Keeping in view the level of contamination and severity of the change, the best possible way to avoid the

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economic losses would be the prevention of OTA contamination but it might not be possible to achieve completely. Instead, it is necessary to adopt measures to detoxify contaminated foods and feed-stuffs for the prevention of deleterious effects and to prevent the buildup of residues when once ingested (Russo *et al.* 2016). In this connection, different strategies have been adopted and the use of adsorbents based on clay minerals is the cost effective and easy method in poultry diet to reduce the systemic bioavailability of mycotoxins. However, clay based adsorbents have shown limited binding efficacy toward fairly non-polar mycotoxins such as OTA, trichothecenes and fumonisins (Avantaggiato *et al.* 2005, Kabak *et al.* 2006, Phillips *et al.* 2008). Therefore, different substances have also been investigated as potential candidate binding agents to alleviate the toxic effect of different mycotoxins by preventing their absorption from the gastrointestinal tract (GIT). Activated carbon (AC), a carbonaceous material having high porosity and large internal surface area; is mainly formed by the heating of organic material in the absence of oxygen (Galvano *et al.* 2001). It is an insoluble powder, used as universal antidote to treat a variety of severe intoxications and also considered among the best currently available substances used for the purification of water (Huwig *et al.* 2001, Lalhruaitluanga *et al.* 2010). AC has the ability to sequester various toxic substances regardless whether they are ionized or not, without being digested in the GIT and acts an absorbent for many gases, drugs and fat soluble substances (Kutlu *et al.* 2001). Several *in-vitro* studies exhibited very high efficacy of AC to adsorb mycotoxins from the aqueous solutions, with an adsorption capacity of up to 120 mg/g (Huwig *et al.* 2001, Avantaggiato *et al.* 2003, Doll *et al.* 2004). Faucet-Marquis *et al.* (2014) reported that during an *in-vitro* study AC efficiently adsorbed OTA, aflatoxin B1 and zearalenone and exhibited a percent adsorption potential of 98.7 ± 0.3, 99.9 ± 0.05 and 99.6 ± 0.4, respectively, in a citrate buffer of pH 3. Plank *et al.* (1990) suggested that the inclusion of 1% AC completely adsorb OTA form an aqueous solution, regardless the pH values ranging from 3–8. Therefore, considering the adsorption ability, the current experimental study was intended to obtain more information about protective efficacy of AC against OTA induced toxicopathological and oxidative changes in the broiler chicken.

Materials and methods

OTA production and quantification

OTA was produced via fermentation of broken wheat grains by the freshly prepared slants of *Aspergillus ochraceus* (CECT: 2948). The sterile substrate (80 g) was placed in flat wide bottom Erlenmeyer flask (1000 mL) and fermentation was carried out at 28 °C for 14 days by the method described by Trenk *et al.* (1971) and Bhatti *et al.* (2018). At day 14, the flasks were autoclaved prior to the extraction of OTA using water: acetonitrile (40:60) and the extracted OTA was quantified via HPLC by the method of Bayman *et al.* (2002).

Birds' housing and feed

1-day-old ($n = 480$) broiler chickens originating from *Salmonella* and *Mycoplasma* free flock were obtained from the commercial hatchery. The chicks were kept on rice hull bedding material under standard environment conditions in the experimental poultry house of the Department of Pathology, University of Agriculture, Faisalabad. A corn and soybean meal based basal broiler feed was formulated without inclusion of antibiotics and toxin binder having 22% total protein and 3100 kcal/kg metabolizable energy (Supplementary Table 1). The birds had free access to feed and water throughout the 42-days of experiment.

The experimental feeds used in the study were prepared by incorporating the known quantity of OTA in the basal as described by Hussain *et al.* (2010). Briefly, the fermented wheat grains were soaked overnight in three fold quantity of chloroform ($CHCl_3$). The mixture was filtered through cotton cheesecloth to separate $CHCl_3$ and the resulting residue was re-suspended in $CHCl_3$ to ensure the complete recovery of OTA. The $CHCl_3$ was evaporated to dryness on a rotary evaporator (Rotavapor® R-210A, Buchi, Switzerland) and concentrated OTA was re-suspended in polyethylene glycol (PEG). The resulting suspension was then evenly mixed initially with 2–3 Kg of basal feed to prepare the mycotoxin feed stock. The desired final OTA concentration in different experimental groups was obtained by further mixing the feed stock with basal feed and the representative sample from each experimental diet was analyzed by HPLC (Prominence™, Shimadzu, Tokyo, Japan) prior to being used for feeding. Following the incorporation of OTA, the candidate binding agent (AC) was added to achieve the desired level in each experimental feed.

Experimental procedure

The experiment was conducted in accordance with all national legislations concerning the protection of animal welfare and the experimental procedures were reviewed and approved by Directorate of Graduate

Table 1. Layout of the experimental design.

Sr. No.	Groups	Number of birds (*n*) in each group	Treatments OTA (mg/kg feed), AC (g/kg feed)
1	Control	30	OTA 0, AC 0
2–4	O1, O2, O3	30	OTA: 0.15, 0.3, 1.0
5–7	A1, A2, A3	30	AC : 2.5, 5.0, 10.0
8–10	O1A1, O2A1, O3A1	30	OTA: 0.15, 0.3, 1.0 AC: 2.5 g/kg with each OTA level
11–13	O1A2, O2A2, O3A2	30	OTA: 0.15, 0.3, 1.0 AC: 5 g/kg with each OTA level
14–16	O1A3, O2A3, O3A3	30	OTA: 0.15, 0.3, 1.0 AC: 10 g/kg with each OTA level

Studies and Animal Ethics Committee of University of Agriculture, Faisalabad, Pakistan. After initial acclimatization period of two days, the chicks were randomly distributed to 16 groups ($n = 30$/group) to be used in the experiment. The different experimental treatments are presented in Table 1. The control group was maintained on basal diet while the groups O1, O2 and O3 were offered basal diet contaminated with OTA at dose rate of 0.15, 0.3 and 1.0 mg/kg feed, respectively. The birds in groups A1, A2 and A3 received basal diet mixed with 2.5, 5.0, 10.0 g AC/kg feed, respectively. Groups O1A1, O2A1 and O3A1 fed diet amended with 2.5 g AC/kg feed along with dietary contamination of 0.15, 0.3 or 1.0 mg OTA/kg, while groups O1A2, O2A2 and O3A2 were fed the three dietary OTA concentrations simultaneously with 5.0 g AC/kg feed. Groups O1A3, O2A3 and O3A3 were offered 10.0 g AC/kg feed against each of the three dietary contamination levels of OTA i.e. 0.15, 0.3 or 1.0 mg/kg.

Parameters studied

Performance parameters

The performance parameters of experimental groups were accessed at day 42 of the experiment by considering the body weights and feed conversion ratio (FCR). Mortality in each group was observed daily and was estimated as percent of total number of birds died throughout the experiment in each group.

Gross and histopathological alterations

At day 42 of age, randomly selected 10 birds from each experimental group were humanly scarified by cervical dislocation. Subjective numerical value varying from 0–3 was attributed to access the presence of each gross lesion on different internal organs; 0= absence, 1–3= presence and severity. The weight of the collected organs (liver and kidney) was recorded to estimate relative internal organ weights (percent of the body weight). The collected tissues from different groups were fixed with neutral buffered formalin 10%, embedded in paraffin and then manually sectioned with a microtome to obtain 4–5 μm-thick paraffin sections for microscopic examination following the method of Bancroft and Gamble (2007).

Serum biochemical parameters

At day 42 of age, prior to humanly scarifying the broiler chickens by cervical dislocation, blood samples from the wing vein were collected without addition of anticoagulant for the collection of serum samples of different experimental groups. Commercially available kits were used to access the serum levels of alanine aminotransferase (ALT) [Merck, France, Catalog # 5.17531.0001], urea [Merck, France, Catalog # 5.17611.0001] and creatinine [Merck, France, Catalog #5.17551.0001. Biuret and bromocresol green dye-binding methods were used to estimate the levels of serum total proteins and albumin, respectively (Davies *et al.* 1984), whereas the concentration of serum globulin was calculated as: [Concentration of serum total proteins–Concentration of serum albumin].

Total antioxidant capacity

Plasma and tissue samples (liver, kidney and breast muscle) were collected at day 42 of the experiment and immediately stored at −80 °C till the estimation of total antioxidant capacity (TAC) of different experimental groups by the method of Erel (2004). Briefly, blank solution was prepared by mixing the sample (5 μL) with reagent-I (200 μL, acetate buffer). Absorbance of the blank solution was measured at 660 nm prior to the addition of reagent II (20 μL, 2,2′-azinobis 3-ethylbenzothiazoline-6-sulfonate) to the blank solution. After the addition, the solution mixture was incubated at 37 °C for 5 min. before the second absorbance of the solution was measured. The TAC of different experimental groups was calculated from the delta absorbance of each sample, by using the plotted standard curve against the different concentrations of the standard.

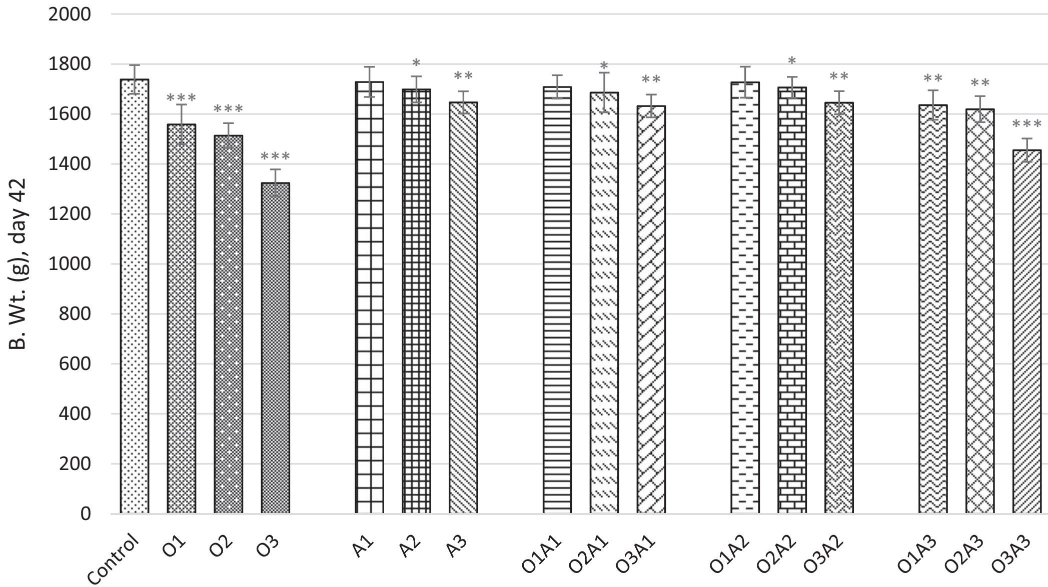

Figure 1. Body weight (g) of broiler chickens at day 42 of the experiment fed different dietary concentrations of OTA and AC alone and in combination. Values shown are means ± SD, differ significantly from control at $^{*}p < 0.05$, $^{**}p < 0.01$, or $^{***}p < 0.001$. Abbreviations: [O1, O2 & O3 = 0.15 mg, 0.3 mg & 1.0 mg OTA/kg feed, respectively], [A1, A2 & A3 = 2.5 g, 5 g, 10 g AC/kg feed, respectively].

Statistical analysis

All the acquired data during the experiment was subjected to analysis of variance (ANOVA) by using MSTATC statistical software and Duncan's multiple range test (DMR) was used to compare different group means. p values < 0.05 was considered as significant.

Results

Body weights and FCR

At day 42, the body weight (Figure 1) of experimental birds offered OTA contaminated diet alone at all levels (O1, O2 and O3) exhibited dose dependent significant decrease ($p < 0.05$) from control, whereas the difference form control was non-significant ($p < 0.05$) in groups A1, O1A1 and O1A2. All remaining experimental groups showed a significant lower ($p < 0.05$) body weight compared to the weight observed in control group. FCR value (Supplementary Figure 1) of all the groups was higher from control with the exception of groups A1, A2 and O1A2 had lower FCR value from the birds in control.

Mortality

Mortality percentage (Figure 2) was highest (23.34%) in birds of group O3, followed by group O2 (13.34%), whereas the lowest percentage (3.34%) was observed in groups A3, O1A1, O2A2, O1A3 and O2A3. However, no mortality was observed in the experimental birds of control group and the birds in groups A1, A2 and O1A2 throughout the course of experiment.

Gross pathological lesions

The experimental birds in control group exhibited the normal gross morphological appearance of internal organs (kidney and liver) during the postmortem examination. Kidneys were restricted to the bony sockets and liver had a normal size, consistency and color. Similar morphological characteristics were also exhibited by the birds in group A1 and A2 whereas the group A3 showed slightly enlarged kidney and liver. In experimental birds fed OTA contaminated diet alone (O1, O2 and O3), the hemorrhagic kidneys were protruding out of the bony sockets. Liver had hemorrhagic surface with friable consistency and pale to yellowish discoloration, representing the dose dependent increase in severity of morphological alterations, being maximum in group O3. In groups simultaneously fed O1 with different dietary concentrations of AC, showed mild to moderate morphological alterations. The severity of these gross lesions was high in the birds offered O2 and O3 simultaneously with three dietary levels of AC however, the severity of the change was lower in comparison to groups offered OTA amended diet alone. The summary of the arbitrary scoring of the gross lesions is shown in Supplementary Table 2.

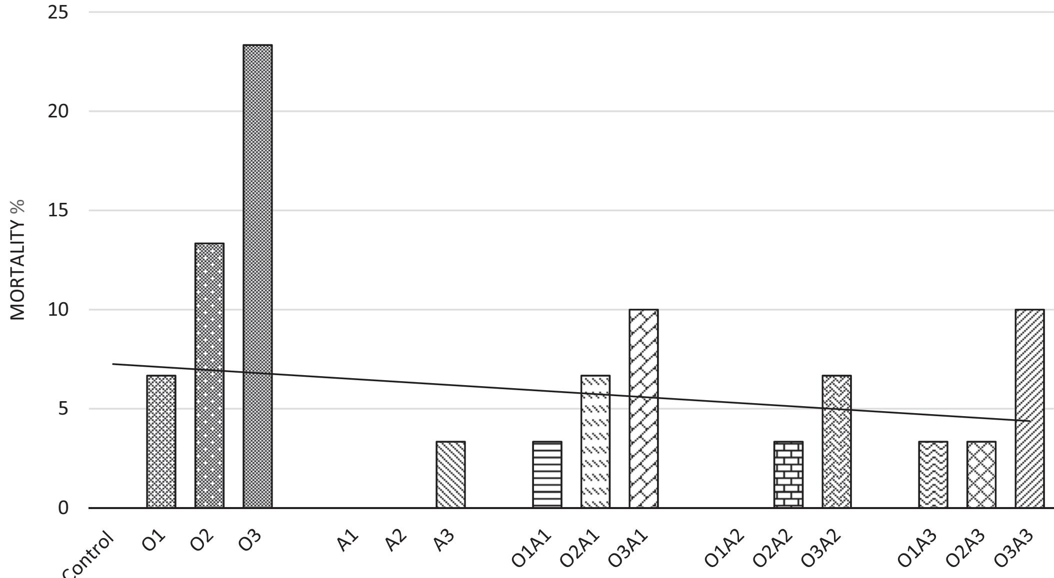

Figure 2. Mortality percentage of broiler chickens fed different dietary concentrations of OTA and AC alone and in combination. Abbreviations are same as described in Figure 1.

Histopathological alterations in kidney and liver

Histopathological examination (Figures 3 and 4) of control group birds exhibited the normal appearance of renal and hepatic parenchyma. The thin layer of epithelial cells lined the clear Bowman's space around the glomeruli and in the surrounded parenchyma tubular epithelial cells had intact nuclei. Hepatocytes exhibited eosinophilic cytoplasm along with prominent round, centrally placed intact nuclei. In the experimental birds fed OTA contaminated diet alone at all tested levels, the renal parenchyma showed dose related increase in severity of degenerative changes as exhibited by prominent cytoplasmic vacuolation along with pyknotic nuclei and detached tubular epithelial cell from the basement membrane. The congested hepatic parenchyma exhibited the necrosis of individual hepatocytes. The groups fed lower concentrations of AC alone (A1 and A2), the histological appearance of kidney and liver was consistent with the birds of control group, however at higher dose (A3) the renal parenchyma was slightly congested. In combination groups the birds offered 0.15 and 0.3 mg OTA/kg feed along with three dietary concentrations of AC (O1A1, O1A2, O1A3, O2A1, O2A2 and O2A3), the renal parenchyma showed slight congestion along with cytoplasmic vacuolation of tubular epithelial cells. The hepatic parenchyma was slightly congested, however around hepatic triads there was no cellular aggregation and sinusoidal spaces were prominent. The birds in groups O3A1, O3A2 and O3A3 exhibited the detachment of renal tubular epithelial cells and there was presence of amorphous homogenous pink material in the renal tubules. The hepatic parenchyma showed vacuolation, congestion and cellular aggregation along with the swelling of the hepatocytes leading to the absence of sinusoidal spaces.

Relative organ weights and serum biochemistry

A significant increased ($p < 0.05$) relative liver and kidney weights (Table 2) were observed in all experimental groups offered different dietary concentration OTA and AC alone or in combination from the relative weights observed in the corresponding control group.

Serum biochemical analysis (Table 2) of groups O1, O2, O3, A3, O3A1 and O3A2 showed a significantly elevated ($p < 0.05$) level of serum urea concentration from control whereas in all other groups the concentration differ non-significantly ($p < 0.05$) from control. A non-significant difference ($p < 0.05$) of serum creatinine level was existed between all the groups from control. Serum ALT levels in groups A1, O1A1, O2A1, O1A2 and O1A3 were non-significant ($p < 0.05$) from control, whereas the level was significantly elevated ($p < 0.05$) in all other experimental groups. A significant lowered ($p < 0.05$) serum level of total proteins and albumin was observed in all experimental groups from control. However, serum globulin level was non-significantly different ($p < 0.05$) in groups A1 and A2 from control whereas in all the remaining groups the serum concentration was significantly decreased ($p < 0.05$) from control.

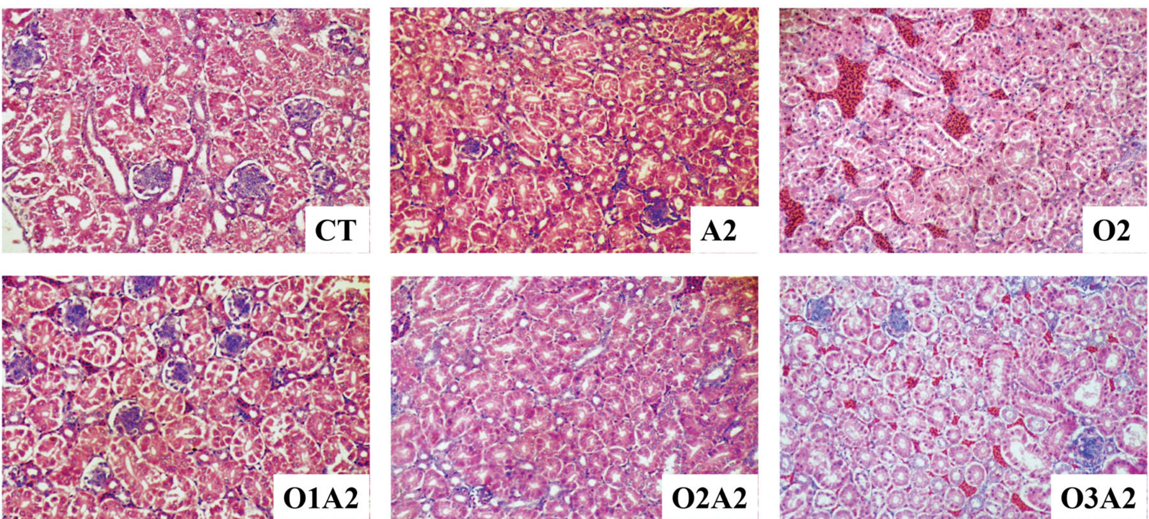

Figure 3. Histopathological alterations in renal parenchyma of the broiler chickens fed different dietary concentrations of OTA and AC alone and in combination with 5.0 g AC/kg feed. Normal histological architecture was observed in control group (CT) and the groups fed basal feed amended with 5.0 g AC/kg feed alone (A2) or simultaneously with 0.15 and 0.3 mg OTA/kg feed (O1A2 and O2A2). The parenchymal tubular cells of the birds fed 0.3 mg OTA/kg feed (O2) exhibited pyknotic tubular epithelial cells and hemorrhagic renal parenchyma. The severity of degenerative change was less in the birds fed dietary combination of OTA (1.0 mg/kg) and AC (5.0 g AC/kg) (O3A2) from the birds in group fed 1.0 mg OTA/kg feed alone (O3). All the tissue sections were stained with H&E (X200).

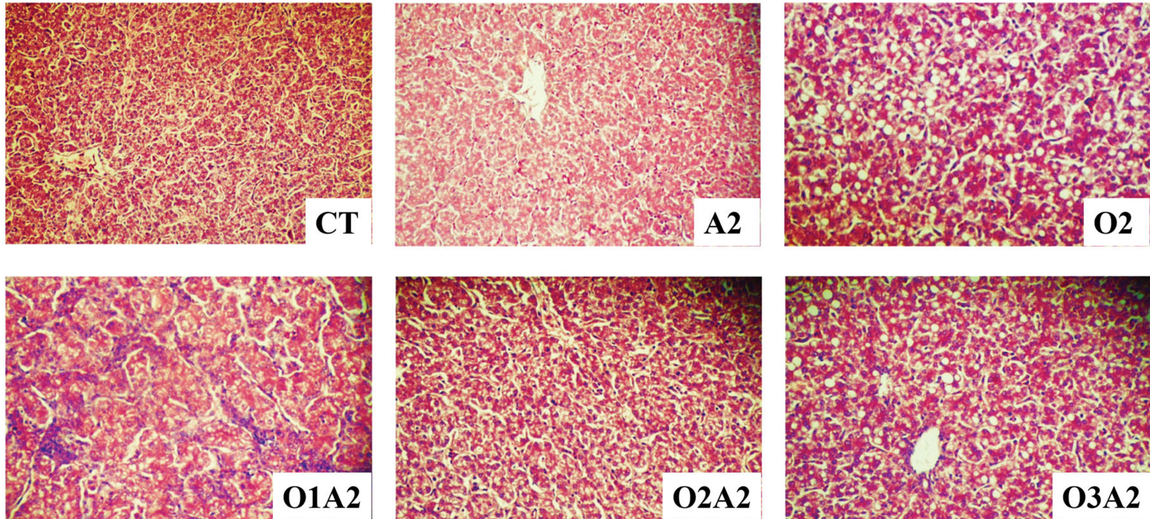

Figure 4. Histopathological alterations in the liver of the broiler chickens fed different dietary concentrations of OTA and AC alone and in combination with 5.0 g AC/kg feed. The hepatic parenchyma of the birds offered basal diet (CT) exhibited a well preserved lobular pattern along with intact centrally placed hepatic nuclei and foamy cytoplasm. The normal histological pattern was also observed in the groups fed 5.0 g AC/kg feed alone (A2). Individual hepatocyte necrosis along with severe vacuolation in hepatic parenchyma was observed in birds fed 0.3 mg OTA/kg alone (O2). In combination groups, moderate cytoplasmic vacuolation and decreased sinusoidal spaces were observed in dose dependent manner being minimum in groups O1A2 and maximum in group O3A2. All the tissue sections were stained with H&E (X200).

Total antioxidant capacity

TAC (Figure 5 (a–d)) was non-significant ($p < 0.05$) in plasma, liver and breast muscle (Figure 5(a,b,d)) of groups A1 and A2 from control, whereas the remaining experimental groups exhibited significant decreased ($p < 0.05$) capacity from control birds. In kidneys, TAC (Figure 5(c)) of group A2 (5.0 g AC/kg feed) was non-significantly different ($p < 0.05$) from control whereas significant decreased ($p < 0.05$) capacity was present in all other groups.

Discussion

Mycotoxins, particularly OTA, are of great concern as they are widely present in feedstuffs around the world

Table 2. Relative weights of liver and kidney and serum biochemical analysis of the broiler chickens fed different dietary concentrations of OTA and AC alone and in combination (Mean ± SD).

	Relative organ weight		Serum biochemical parameters					
Group	Liver	Kidney	Urea (mg/dl)	Creatinine (mg/dl)	ALT (IU/µL)	Albumin (g/dl)	Globulin (g/dl)	Total Protein (g/dl)
Control	2.37 ± 0.18 [g]	0.46 ± 0.06 [g]	12.20 ± 1.88 [f]	0.30 ± 0.02	16.10 ± 2.52 [f]	3.66 ± 0.06 [a]	1.50 ± 0.10 [a]	5.15 ± 0.06 [a]
O1	3.18 ± 0.28 [bcd]	1.09 ± 0.13 [c]	15.94 ± 1.62 [cd]	0.33 ± 0.01	29.42 ± 3.65 [c]	2.96 ± 0.04 [i]	0.63 ± 0.06 [g]	3.60 ± 0.06 [j]
O2	3.34 ± 0.46 [bc]	1.15 ± 0.10 [c]	17.47 ± 2.78 [bc]	0.37 ± 0.02	33.67 ± 3.51 [b]	2.81 ± 0.06 [j]	0.42 ± 0.24 [h]	3.23 ± 0.19 [k]
O3	4.23 ± 0.47 [a]	1.33 ± 0.02 [a]	27.64 ± 2.20 [a]	0.48 ± 0.02	41.96 ± 4.32 [a]	2.15 ± 0.05 [m]	0.15 ± 0.07 [i]	2.30 ± 0.04 [l]
A1	2.90 ± 0.05 [def]	0.69 ± 0.03 [ef]	13.20 ± 1.94 [ef]	0.31 ± 0.01	19.53 ± 2.15 [def]	3.50 ± 0.05 [b]	1.46 ± 0.05 [ab]	4.95 ± 0.06 [b]
A2	2.87 ± 0.14 [def]	0.69 ± 0.07 [ef]	14.39 ± 1.97 [def]	0.30 ± 0.01	21.57 ± 3.19 [de]	3.42 ± 0.05 [c]	1.44 ± 0.08 [ab]	4.86 ± 0.06 [c]
A3	2.90 ± 0.27 [def]	0.64 ± 0.01 [f]	19.28 ± 2.57 [b]	0.27 ± 0.02	22.75 ± 2.55 [d]	3.40 ± 0.04 [cd]	0.87 ± 0.06 [f]	4.27 ± 0.06 [ef]
O1A1	2.77 ± 0.25 [f]	0.92 ± 0.06 [d]	12.92 ± 1.98 [ef]	0.30 ± 0.01	18.00 ± 3.20 [ef]	3.22 ± 0.04 [f]	1.09 ± 0.07 [cd]	4.32 ± 0.04 [de]
O2A1	2.87 ± 0.07 [def]	0.98 ± 0.01 [d]	14.18 ± 2.12 [def]	0.32 ± 0.03	20.76 ± 2.80 [def]	3.04 ± 0.03 [h]	0.96 ± 0.04 [ef]	4.00 ± 0.05 [g]
O3A1	3.04 ± 0.07 [cdef]	1.12 ± 0.02 [c]	17.85 ± 2.19 [bc]	0.40 ± 0.01	28.14 ± 2.99 [c]	2.63 ± 0.04 [l]	1.12 ± 0.09 [c]	3.75 ± 0.09 [i]
O1A2	2.80 ± 0.07 [f]	0.90 ± 0.08 [d]	12.77 ± 1.54 [f]	0.31 ± 0.01	20.69 ± 3.10 [def]	3.36 ± 0.04 [de]	1.01 ± 0.07 [de]	4.37 ± 0.07 [d]
O2A2	2.82 ± 0.41 [ef]	0.91 ± 0.01 [d]	13.42 ± 2.00 [def]	0.30 ± 0.01	21.94 ± 3.12 [de]	3.24 ± 0.04 [f]	0.97 ± 0.03 [ef]	4.22 ± 0.04 [f]
O3A2	3.14 ± 0.15 [bcde]	1.11 ± 0.09 [c]	15.56 ± 1.50 [cde]	0.39 ± 0.01	26.66 ± 2.28 [c]	2.71 ± 0.04 [k]	1.35 ± 0.07 [b]	4.06 ± 0.04 [g]
O1A3	2.78 ± 0.14 [f]	0.73 ± 0.02 [e]	13.17 ± 1.75 [ef]	0.32 ± 0.01	19.45 ± 2.68 [def]	3.31 ± 0.04 [e]	0.87 ± 0.07 [f]	4.19 ± 0.04 [f]
O2A3	2.85 ± 0.33 [def]	0.96 ± 0.03 [d]	12.34 ± 1.77 [f]	0.31 ± 0.01	21.24 ± 2.24 [de]	3.11 ± 0.04 [g]	0.97 ± 0.03 [ef]	4.08 ± 0.04 [g]
O3A3	3.38 ± 0.14 [b]	1.24 ± 0.03 [b]	13.29 ± 2.06 [ef]	0.44 ± 0.02	30.13 ± 2.44 [c]	2.78 ± 0.03 [j]	1.12 ± 0.07 [cd]	3.89 ± 0.05 [h]

[a-g]The values in each column with no common superscript letter are significantly different at $p < 0.05$.
The values without superscript letter in a column are non-significant from each other.
Abbreviation: [O1, O2 & O3 = 0.15 mg, 0.3 mg & 1.0 mg OTA/kg feed, respectively], [A1, A2 & A3 = 2.5 g, 5 g, 10 g AC/kg feed, respectively].

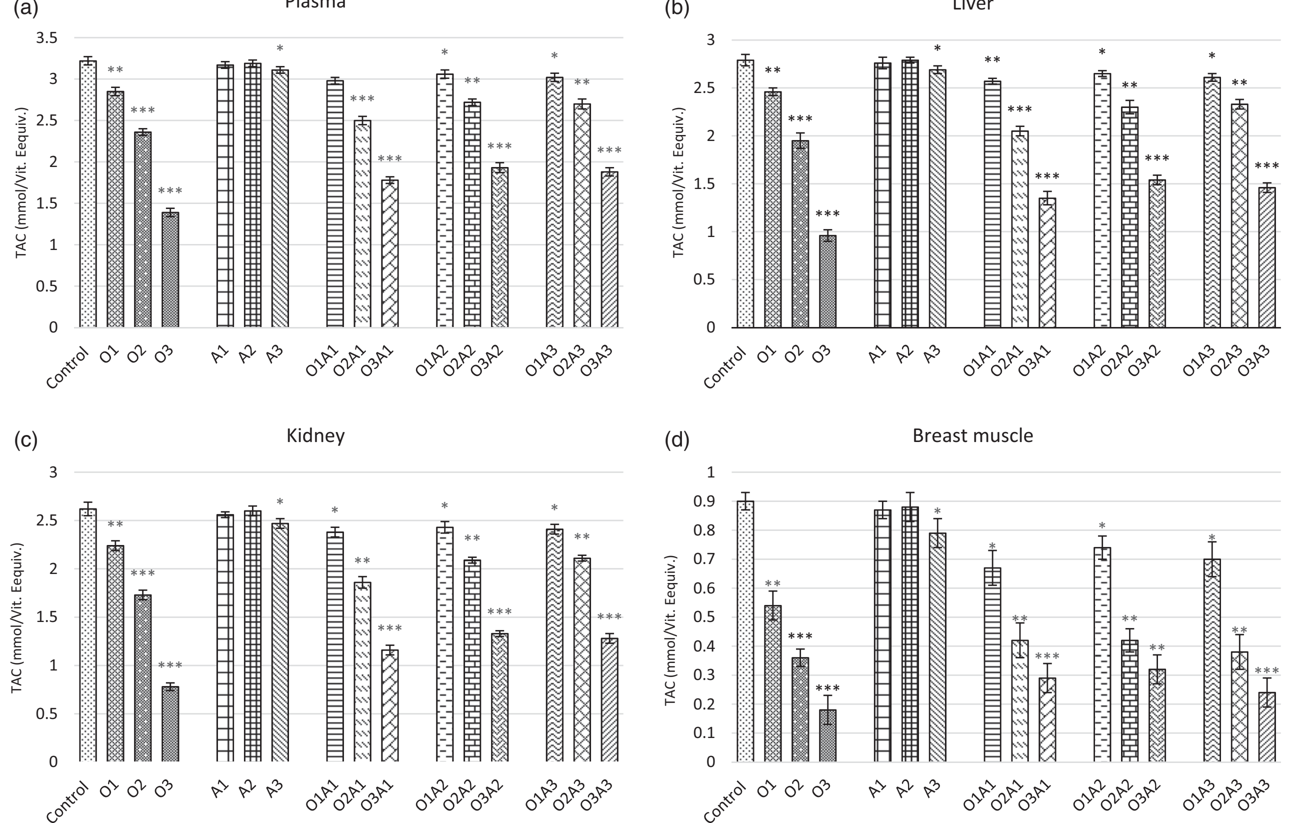

Figure 5. (a–d) Effect of different dietary concentrations of OTA and AC alone and in combination on the TAC of broiler chickens. Values shown are means ± SD, differ significantly from control at $^{*}p < 0.05$, $^{**}p < 0.01$, or $^{***}p < 0.001$. Abbreviations are same as described in Figure 1.

and possess a permanent challenge for the poultry industry; affect the health status even in very low concentrations and results in severe economic losses. There have been some attempts to prevent the systemic bioavailability of mycotoxins from GIT by using different entero-sorbents (Garcia *et al*. 2003, Trailovic *et al*. 2013, Pfohl-Leszkowicz *et al*. 2015). The use of in-feed clay minerals or yeast cell wall as mycotoxin adsorbents to protect the bird health and productivity has markedly increased in recent years throughout the world. However, due to limitations in their efficacy to bind various mycotoxins in the GIT, the focus of the current experimental study was to evaluate the potential adsorbing efficacy of AC against graded concentrations of OTA in the broiler chickens to explore the rate and extent of its absorption to prevent the systemic toxicity.

Based on the use of very fine black powder of AC as universal antidote to treat different toxicities and also during *in-vitro* studies where it has shown more than 90% adsorption efficacy against OTA (Sands *et al*. 1976, Dalvi & McGowan 1984, Jindal & MahiPal 1999), the dietary incorporation of 2.5 and 5.0 g AC/kg feed alone (A1 and A2) in the present study did not significantly ($p < 0.05$) alter the health and growth performance of the broiler chickens from the birds in control group. However, the difference from control was significant ($p < 0.05$) in the birds fed diet amended with 10 g AC/kg (A3), considering the health and growth performance parameters. At higher dietary level of AC, the reduced body weight might be due to blackening of the feed which negatively influence the palatability and also being nonspecific in nature, AC might result in binding of many essential nutrients from feed thus reducing its nutritive value (Ramos *et al*. 1996, Huwig *et al*. 2001).

In current experimental study, dietary OTA at all levels induced a dose dependent negative impact on body weight, FCR and internal organs relative weights. The gross morphological and histopathological alterations in internal organs were also increased in dose related manner. OTA intoxication at all levels significantly ($p < 0.05$) altered the serum proteins, enzymes and TAC of the birds. The injurious effects of OTA concentration used in the present study were consistent with earlier reports of Koynarski *et al*. (2007), Elaroussi *et al*. (2006) and Verma *et al*. (2004). The reduced weight gain, as observed in the present study, might be due to decreased feed intake and poor FCR (Elaroussi *et al*. 2006). Kubena *et al*. (1990) suggested that the decrease weight gain and FCR could be due to the impaired protein synthesis because of competitive inhibitory effect of phenylalanine moiety of OTA; prevent the attachment of amino acid phenylalanine to Phenylalanine-tRNA synthetase. OTA intoxication induce early hepatotoxicity (Qi *et al*. 2015) and the resultant hypoproteinemia and decrease concentration of somatotropic hormones resulted in reduced body weight and feed consumption (Elaroussi *et al*. 2006). The increased relative weight of internal organs (liver and kidney) might be due to the damage caused by OTA intoxication as both are the major organs involved in OTA biotransformation and removal from the body (Fuchs *et al*. 1988, Qi *et al*. 2015).

In combination groups, feeding graded concentrations of OTA simultaneously with dietary levels of AC, body weight and FCR values suggested partial adsorption ability of AC against OTA. However, in comparison to groups O1, O2 and O3 the observed reduced body weight and increased FCR values were positively influenced in combination groups. Similarly, lowest mortality percentage was observed in combination groups from groups fed OTA contaminated diet alone at all levels. The groups offered lower dietary contamination of OTA (0.15 and 0.3 mg/kg) simultaneously with 2.5 and 5.0 g AC/kg feed, the provided protection of AC was accessed by observing the decreased severity of change in the relative organ weights, gross and microscopic alterations in the internal organs, serum biochemical analysis and antioxidant status of the broiler chickens. However, this protective response was absent against 1.0 mg OTA/kg feed, although the improvement in the parameters was obvious compared to the birds offered OTA alone. It is to mention that the adsorption efficacy of AC against OTA was accessed by observing the non-significant differences ($p < 0.05$) of combination groups with the control. Previously, the addition of extremely porous carbon derived from coconut shell to the broiler feed significantly reduced the OTA (0.4 mg/kg) induced injurious effects on various performance and serum biochemical parameters. However, dietary supplementation of charcoal at dose rate of 10 000 ppm did not significantly decreased the OTA (4 ppm) induced toxic effects in Leghorn chicks (Rotter *et al*. 1989). Wang *et al*. (2006) reported that supplementation with 1.0% AC did not improve the serum biochemical alterations in broiler chickens fed 10 μg OTA/kg feed. An enhanced feed consumption and body weight of broiler chickens was observed by simultaneous use of dietary AC against aflatoxins compared to the birds offered dietary aflatoxins alone (Dalvi & McGowan 1984, Jindal *et al*. 1994). However, Edrington *et al*. (1997) stated that the dietary inclusion of 0.5% superactivated charcoal to male broilers for 21 days, did not significantly alter the deleterious effect of aflatoxin on feed to gain ratio,

weight gain and gross lesions compared to the birds fed aflatoxins alone. In turkey poults the aflatoxin induced alterations in urea nitrogen and glucose concentrations were ameliorated by the use of AC (Edrington *et al*. 1996). In broiler chickens the simultaneous use of AC along with yeast and zeolite effectively reduced the aflatoxin B1 induced toxicities (Khadem *et al*. 2012).

Many hypotheses exist on the main toxic mechanism of OTA; phenylalanine moiety of OTA interferes with synthesis of DNA, RNA and protein by the competitive inhibition of phenylalanine in enzyme-catalyzed reaction and also inhibit the process of gluconeogenesis by interfering with mRNA responsible for the coding of key enzyme phosphoenolpyruvate carboxykinase (Murugesan *et al*. 2015). The formation of reactive oxygen species contribute to the lipid peroxidation and thereby compromising the permeability and solubility of the cell membrane (Mazur-Kuśnirek *et al*. 2019). However, the exact knowledge about the target cells responsible for the toxic consequences after the long-term exposure with OTA is still unknown (Koszegi & Poor 2016). The use of adsorbents can enhance the elimination of OTA from the body but at the same time the non-polar nature of OTA is the major factor to effect the adsorption ability of most of the binders (Sirhan *et al*. 2012) and no single binder up till now has shown equal adsorption efficacy against different types of mycotoxins. The shape, charge distribution, polarity and solubility of mycotoxin significantly influence the adsorption capacity of the binder (Huwig *et al*. 2001, Avantaggiato *et al*. 2005). Although, during *in-vitro* studies AC exhibited more than 90% adsorption efficacy against OTA but the expected adsorption ability during *in-vivo* studies is very limited (Rotter *et al*. 1989, Plank *et al*. 1990). The adsorption efficacy of AC mainly depends on its pore size, surface area and chemical nature of the source (Diamadopoulos *et al*. 1992). The biochar prepared at higher temperature (800 °C) exhibited higher OTA adsorption capacities and faster adsorption kinetics indicating the influence of pyrolysis temperatures on the adsorption characteristics (Ahmadou *et al*. 2019). The major protective mechanism of AC is probably through chemisorption and the sequestrating of mycotoxin in the GIT (Edrington *et al*. 1997). AC acts as a nonspecific insoluble carrier thereby reduce the bioavailability of OTA after being ingested (Rosa *et al*. 2001). The nonspecific binding property of AC could reduce the nutrients availability and also impaired the nutritive value of feed especially if in relation to the mycotoxin its concentration is much high in the diet. The deterioration observed at higher dietary concentrations of AC could be the indication of reduced absorption of nutrients form GIT (Kutlu *et al*. 2001, Avantaggiato *et al*. 2004).

Conclusions

It could be concluded that dietary OTA at all the tested levels (0.15, 0.3 and 1.0 mg/kg) negatively influence the health status of the broiler chickens in dose related manner. The observed significant differences ($p < 0.05$) of various combination groups from control exhibited that the AC reduced the intensity of deleterious effects induced by 0.15 and 0.3 mg OTA/kg feed on the studied parameters. However, the adsorption ability of AC was absent against 1.0 mg OTA/kg feed. The use of higher concentration of AC (10 g/kg) alone in the diet also impaired the health status of the broiler chickens.

Acknowledgements

The authors highly acknowledge the Higher Education Commission of Pakistan for funding this study to first author under HEC Indigenous PhD fellowship program batch VII.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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