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In Vivo Detoxification of Ochratoxin A by Highly Porous Magnetic Nanocomposites Prepared from Coconut Shell

ABSTRACT

The current study was conducted to elucidate the clinical effect of ochratoxin A in *Gallus gallus domesticus* and to ameliorate its toxic effects by the development and characterization of highly porous carbon-based adsorbent derived from coconut shell. A series of experiments were performed on one day-old chicks (Group A to F). Clinical signs of the positive control (group B fed on 400 ppb ochratoxin A contaminated feed) included depression, diarrhea, increased water intake, low body weight, high degree of genotoxicity, swollen and hemorrhagic kidneys and liver.

Serum alanine amino transferase (ALT), alkaline phosphatase (ALP), creatinine and urea concentration significantly increased while albumin, globulin and total proteins were found significantly low. All these lead to increased mortality.

Among adsorbents treated groups, chickens in group C (0.3% of the prepared adsorbent mixed in OTA contaminated feed) and D (0.6% of the prepared adsorbent mixed in Ochratoxin A (OTA) contaminated feed) showed very low pathological effects while group E (0.9% of the prepared adsorbent mixed in OTA contaminated feed) and F (1.2% of the prepared adsorbent mixed in OTA contaminated feed) showed normal behavior, blood biochemistry and well maintained histological structure like that in group A. As a whole, the adsorbents treated groups fed with ochratoxin A contaminated feed, supplemented with different levels of the prepared adsorbent helped to ameliorate the toxic effects of OTA and nearly showed normal clinical signs.

INTRODUCTION

Ochratoxins are a group of mycotoxins produced as secondary metabolites by toxigenic mycobiota. Structurally, all the ochratoxins consist of derivatives of polyketide dihydrocoumarin moiety linked through the 7- carbon group to L-β-phenylalanine by an amide bond (Vander *et al.*, 2001). Avian ochratoxicosis is a disease that affects birds, which the causative agent is ochratoxins. Sensitivity to ochratoxin A (OTA) may vary among different species of birds. Vulnerability to OTA induced diseases may also vary depending on cell type and sex of a species, for instance renal tissue damage caused by ochratoxin A is severe when compared to the rest of tissue damage. OTA contamination in poultry feed results in growth retardation, low efficiency and feed intake (Peckham *et al.*, 1971) and high rate of mortality (Elaroussi *et al.*, 2006). OTA is also nephrotoxic and causes gross lesions and severe renal hemorrhages as well as hepatic tissue hemorrhage that finally leads to impaired renal function. Besides nephropathic effects, ochratoxin A have negative effects on the liver. Several authors have reported hepatotoxicity, cytoplasmic vacuolation of hepatocytes and fatty deposition in the liver of poultry chicks when fed with ochratoxin A contaminated feed (Elaroussi *et al.*, 2002).



Over the former few decades, literature has been published to reveal the approaches involved in ochratoxin A carcinogenicity and toxicity (Schilter *et al.*, 2005). There has been significant discussion about the genotoxic and carcinogenic potential of OTA (Kuiper-Goodman & Scott 1989). Numerous researchers and proficient groups have determined that OTA is genotoxic as well as nephrotoxic (Pfohl-Leszkowicz & Manderville 2012). Control and prevention of ochratoxin contamination is necessary. If it still occurs, then it is compulsory to detoxify it prior to usage. Various chemical, physical and biological measures and treatments have been taken into account for ochratoxin detoxification.

One of the effective methods to treat mycotoxicosis in animals and poultry birds is the application of adsorbents (Medina *et al.*, 2007). Over the last few decades, there has been a constant growing attention and exploration of efforts in the manufacturing, functionalization, characterization, molecular composition and design of extremely porous carbon-based adsorbents plus their application against mycotoxin (Arenillas *et al.*, 2005). Although activated carbon has high surface area and can effectively adsorb mycotoxins in the gastrointestinal tract of chicks but also causes dehydration and minerals depletion. Also, it cannot easily be collected from feces after treatment and will cause secondary environmental problems as the adsorbed mycotoxin will reach the human body through food chain. Magnetic activated carbon on the other hand has comparatively less surface area when compared to activated carbon and do not cause dehydration and mineral deficiency in chicks. Also due to magnetic character it can be easily collected from feces through magnetic processes (Khan & Zahoor 2014).

Keeping in view the importance of ochratoxin A in poultry industry, the present study was designed to determine the *in vivo* efficacy of the novel adsorbent prepared from coconut shell in broiler chicks.

MATERIALS AND METHODS

Preparation and characterization of adsorbent

In this study a novel magnetic adsorbent from coconut shell was prepared by following the methods of Khan *et al.* (2014) with slight modification. Coconut shell was utilized as bulk material for the preparation of carbon-based adsorbents. The material was crushed and soaked in ethanolic $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ (10% w/v). When

half of an hour has passed, the biomass is taken out and dried at room temperature for about 24 hours. The dried materials were then passed through pyrolysis fumigation for 48 hours for pyrolysis. The pyrolysis treated biomass was then shifted to an oven for drying at 100 °C for 5 hours. After that material was taken out from the oven and shifted to a specially designed chamber consisting of a stainless steel container comprising of electric heater, a wire gauze, inlet, exhaust outlet and supply of nitrogen gas (to provide inert environment) and was charred. Temperature in the chamber was 500 °C, which was set in such a manner that there was 10 °C increase in temperature/min. The material was kept in the chamber for 2 hours to accomplish the process. When the processed sample was subjected to magnetic bar all the sample contents were attracted toward it. This clearly indicated that formation of iron oxide has occurred. After the preparation the adsorbent was treated with different concentrations of HNO_3 solution to remove 50% of the iron contents. The highly porous adsorbent prepared was then characterized by different instrumental techniques (surface area analyzer, FTIR (Fourier-transform infrared spectroscopy), SEM (Scanning electron microscope), XRD (X-ray diffraction), TG/DTA (Thermogravimetric/differential thermal analysis), EDX (Energy-dispersive X-ray spectroscopy) etc.).

Experimental protocol for induction of ochratoxin A in broiler chicks

A total of 600, one day old broiler chicks (*Gallus gallus domesticus*) were purchased from a local hatchery (Rawalpendi, Pakistan) and were reared in well managed and hygienic conditions. All these chicks were divided in six equal groups (A-F) containing 100 chicks in each and access to fresh water was given *ad libitum*.

Chicks in group A were provided with ochratoxin A free diet during the whole period of the experiment and served as (negative control). Chicks in group B were provided with ochratoxin A (400 µg/kg) contaminated feed during the whole period of the experiment and served as (positive control). Ochratoxin A was produced on maize by following the protocols of Shotwell *et al.* (1966). The details of the method were as follow:

One liter round bottom flask was taken and 50 g of crushed maize along with 25 ml of distilled water was added to it. The flask (containing maize and water) was shaken well and then placed at room temperature for 2 hours. About 3 ml of spores were taken from PDA slant and were inoculated in the flask and then



incubation was carried at 28 °C for 5 days. Before loading spores, the flask was autoclaved at 121 °C for 20 min and allowed to cool at room temperature. The flask was autoclaved after five days at 121 °C for 20 minutes. The flask content was then cooled, mixed and the level of ochratoxin A was calculated by applying HPLC (Hitachi model L-200) analysis.

Chicks in group C, D, E and F were kept on ochratoxin A (400µg/kg) contaminated feed mixed with 0.3%, 0.6%, 0.9%, 1.2% of the prepared adsorbent respectively throughout the duration of the study.

The mixing ochratoxin A with feed, powdered maize having the required quantity of ochratoxin A was weighed and saturated in 3 parts of chloroform for 24 hours. Subsequently the filtration of the whole material was done by using cloth filter. Then chloroform was evaporated, and the saturated solution was obtained in glass funnel. After that 100 ml Poly Ethylene Glycol was added to the filtrate. Half kg of the poultry feed was separated, and the prepared solution was mixed in it. Ochratoxin A mixed feed was further mixed to 2 kg of feed and by this way the required quantity of contaminated poultry feed was prepared.

Observation and analysis

Several parameters such as genotoxicity, histopathology, gross lesions, organ enlargement, total body weight, organ weight, mortality and clinical signs of chicks were noted at the end of each week. Decrease or increase in body weight or in organ weight was measured through digital balance.

Observations of gross lesions, hemorrhages and morphological alterations were carried through visual examination, because these medical characteristics are easily observed through naked eye.

On the other hand, blood serum biochemistry was analyzed by slaughtering three individual chicks from each group at the end of the 1st to the 6th week of the experiment.

Data was statistically analyzed for mean, standard error of mean, standard deviation, descriptive statistics, all pairwise comparison tests and completely randomized tests by using software STATISTIX 8.1.

Single cell gel electrophoresis (SCGE) / Comet Assay

Genotoxic effect of OTA was determined by following the protocols of Singh *et al.* (1988) with slight modifications. The blood was collected in a 5ml sterilized syringes from the brachial vein of the selected chicks. For genotoxicity of kidney and liver, about 1g of each tissue was grinded separately through grinder machine and about 5-6 ml of phosphate buffer saline (PBS) was added to the grinded tissues to obtain the solution.

To the single coated slide, 75 µL of low melting point agarose (LMPA, 1.5%) mixed with 15 µL of blood was added. A cover slip was placed on it and the slide was put on an ice pack for ~5 to 10 minutes. The cover slip was removed and a third LMPA layer of 85 µL was added to the same slide. After 10 min on the ice pack, the coverslip was removed and slides were shifted to freshly made Lysing solution poured in a glass tray overnight at 4 °C. After electrophoresis the slides were rinsed with a Neutralization Buffer and stained with 70µL DNA specific fluorescent dye Acridine Orange (20 µg/ml). Before observing the slides, the excess of water on the edges and back of the slides was blotted away. From every slide 100 cells were randomly selected and images (Figure 1) were taken at 400x by using fluorescent microscope (Nikon Eclipse 80 i) equipped

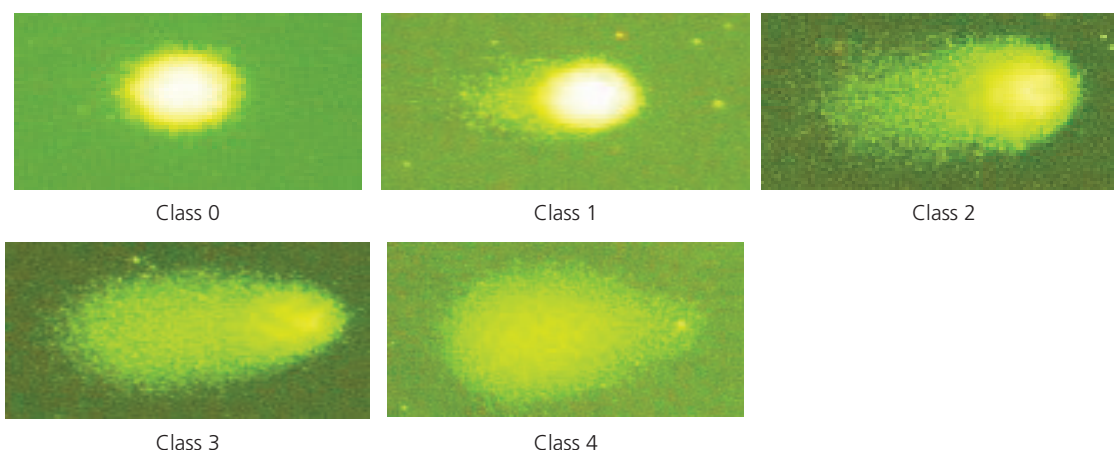


Figure 1 – Different comet classes (0-4) and level of DNA damage.



with an excitation filter of 450-490 nm. Comet tail lengths (consisting of nuclear region and tail) were scored visually into 5 Comet Classes:

Comet Class 0 (no damage, hence no tail), Comet Class 1 (tail up to 1.5 times the diameter of the comet nucleus), Class 2 (tail 1.5–2.0 times the diameter of the comet nucleus), Class 3 (tail 2.0–2.5 times the diameter of the comet nucleus) and Class 4 (maximally damaged with total DNA in its tail).

A final overall total comet score for all 100 cells was obtained by summing up the number of cells in each class times the class number, giving a rating between 0-4 i.e.

$TCS = 0(n) + 1(n) + 2(n) + 3(n) + 4(n)$, Where (n) indicate the number of cells in each class.

Tissue processing for histological evaluation

For histological evaluation the tissues were processed manually according to the procedures of laboratory methods in histotechnology (Prophet & Edna 1992).

After dissecting the selected broiler chick, kidney and liver tissue samples were immediately transferred to 10% neutrally buffered formalin and remained in fixative for a minimum of 48 hours. Then these tissues were dehydrated in different concentrations of ethanol solution and finally transferred to a clearing solution. The dehydrated and cleared tissues were infiltrated with melted paraffin and embedded in stainless steel (S.S) moulds. The moulds were then placed in a freezer and when completely solidified, the tissue blocks were removed. The tissues were sectioned through a rotary microtome and stained with Harris hematoxylin and eosin-phloxine solution. Photomicrographs of tissues sections were taken on a digital camera attached to the microscope.

Biochemistry of blood

Serum biochemical parameters such as alanine transferase (ALT), serum alkaline phosphatase (ALT), Serum albumen, total proteins, globulin, serum urea and creatinine were determined by using commercially available kits of RANDOX.

Clinical signs

Clinical signs and behavior of poultry chicks (attentiveness, attraction to water and feed, and consistency of feces) were analyzed by score from 0 to 3 based upon (0) absences, (1) minor, (2) average and

(3) extreme. These interpretations and observations were kept in record at end of each week of experiment.

RESULTS AND DISCUSSION

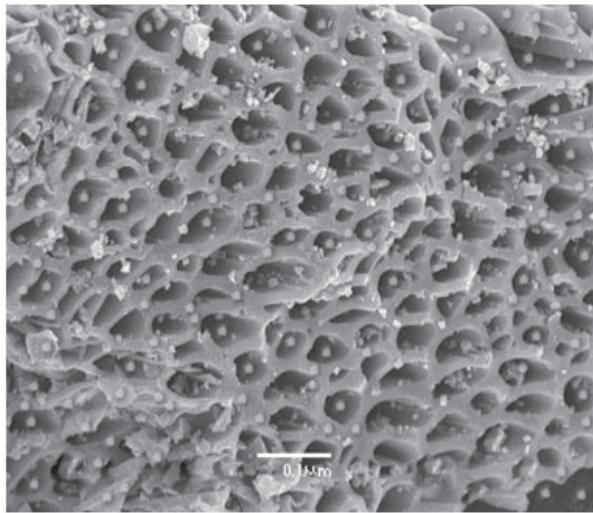
Highly porous carbon nanocomposite

The prepared magnetic adsorbent has a high Langmuir surface area of 989.12 m²/g with 50% magnetic character as well. The adsorbent was characterized by different instrumental techniques (Figure 2) and used in *in-vitro* for the detoxification of ochratoxin A (our unpublished paper). SEM picture confirmed that the excess of deposited Iron oxide was removed when treated with HNO₃ thus leaving the nano-pores in the prepared sample. The pores size was noted below 100 nm however the remaining of iron oxide particles after treatment with HNO₃ solution as white patches can also be seen in the highly porous carbon nano-composite prepared from coconut shell. The surface morphology of Iron oxide nanoparticles was carried out by Sneha *et al.* (2015) and confirmed the porous nature of the product through SEM.

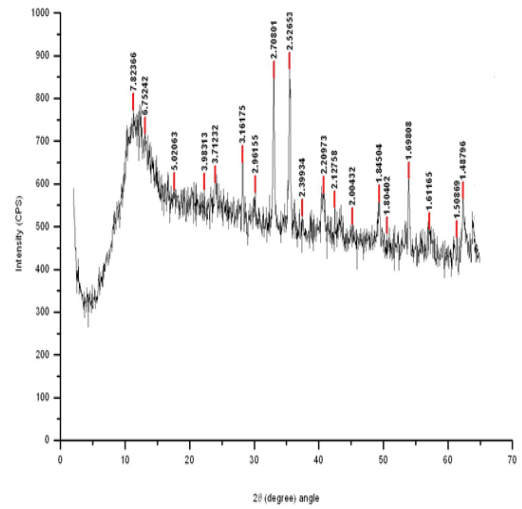
The XRD patterns have indicated that iron oxide (magnetite) is deposited on the activated carbon, while EDX showed the peaks of Fe and O. The EDX pattern in figure 2 shows Fe 0.7, 2.6, 6.5 and 7 KeV which confirms the presence of iron in the composite structure. In our previous work similar EDX spectra of the composite was obtained (Zahoor & Khan, 2014). The TG/DTA pattern in figure 2 shows a gradual loss of weight up to 390 °C. An abrupt fall in loss of weight was noted from 400 °C to 595 °C, whereas no loss of weight was observed above 600 °C.

The mid and far IR spectra of highly porous carbon nano-composite, prepared from coconut shell, are presented in figure 2. The FTIR spectrum exhibit band at 3412 cm⁻¹ showing stretching vibration of O-H whereas 2600cm⁻¹ representing the aliphatic C-H. The bands between 1200 to 1000 cm⁻¹ can be attributed to C=O and C=C respectively (Wang *et al.*, 2009). Other significant bands at 1002.98 and 1080 and 1190 cm⁻¹ are ascribed to C-O stretching as reported in earlier studies (Hadi *et al.*, 2015). The band at 587 cm⁻¹ corresponds to stretching of Fe-O deposited in the pores and surface of the prepared adsorbent from coconut shell as reported previously (Sneha *et al.*, 2015, Zahoor & Khan, 2015).

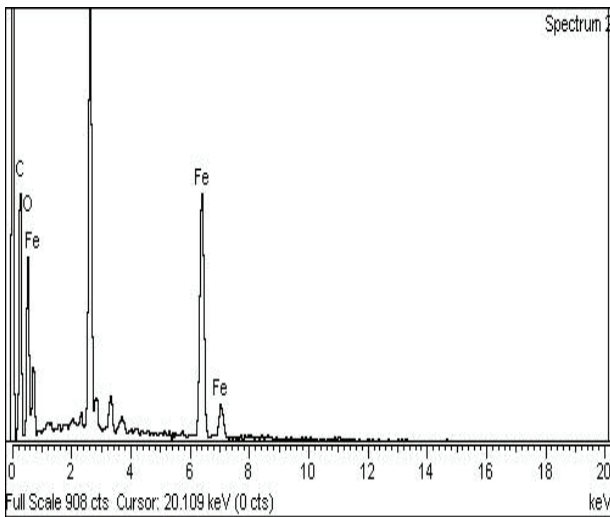
The novel adsorbent was then used for the *in-vivo* detoxification of ochratoxin A. The *in-vivo* results are described below.



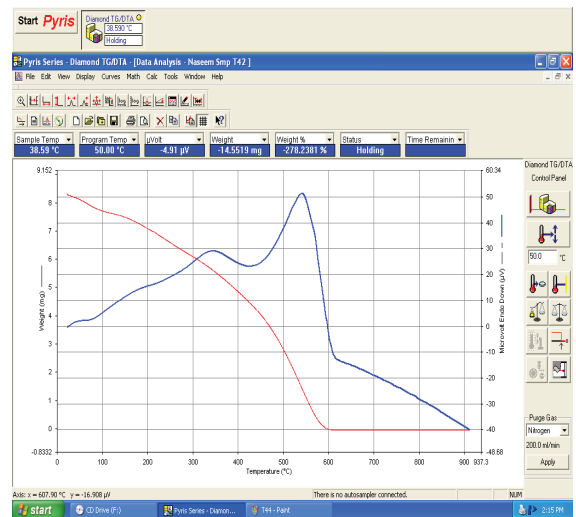
ESM



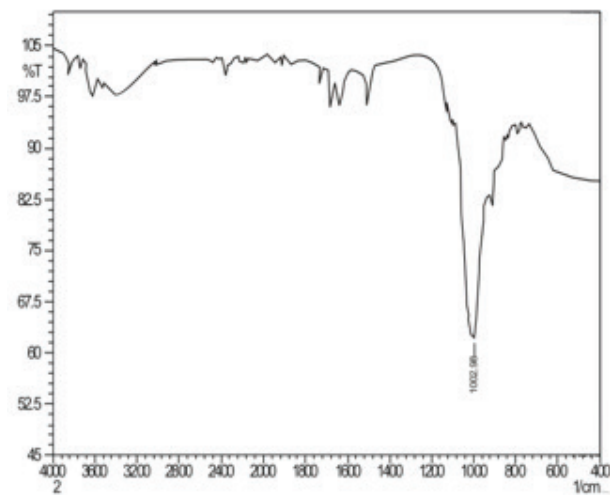
XRD



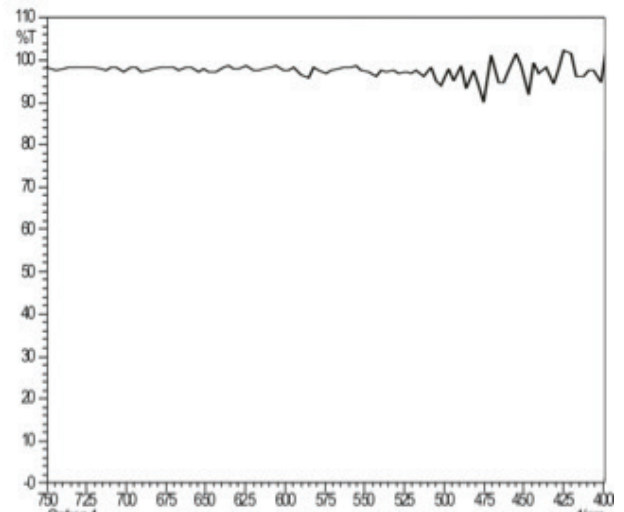
EDX



TG/DTA



Mid FTIR spectra



Far FTIR spectra

Figure 2 – Photomicrographs showing characterization of highly porous Iron oxide carbon nano-composite prepared from coconut shell.



Ochratoxin A induced genotoxicity and its detoxification in broiler chicks

Blood, kidney and liver tissue samples of broiler chicks from each group were taken and treated to estimate DNA damage caused by ochratoxin (1st week to the 6th week). The DNA damage observed in the blood, kidney and liver tissue samples in group B and C was significantly greater than that estimated in group A, E and F (Table 1s to 6s). The blood, kidney and liver samples in group B and C showed higher mean frequency of total comet score (TCS) and comet class per 100 cells as compared with those from group A, E and F. Besides value of comet class 0 was highest in the blood in group A (negative control) while lowest mean values of comet class 0 were observed in the blood in group B birds (positive control). During the whole experimental period blood samples of broilers in group A (negative control) showed maximum of class (0) and minimum score of class (4). Thus, maximum mean value of class (0) is shown by group A. Mean value of comet class (4) in blood samples of broilers in group B (positive control) was low during the first week but with the increase in exposure duration, there was an increase in score of class (4). The increase in score of class (4) is indication by high degree of genotoxicity. The value of TCS at the end of the 6th week was 221.97±9.9. Thus, the maximum total comet score (TCS) and high degree of genotoxicity was shown by

the chickens in group B followed by group C. The value of TCS of chickens in group A, E and F was 24.94±4, 43.06±7.6 and 26.96±11.2 respectively at the end of the last week of the experiment which represents least TCS. The sequence for total comet score (TCS) in the blood sample of different groups at the end of the experiment was in the following order B > group C > group D > group E > group F > group A. Thus, the higher total comet score is shown by group B.

Likewise, liver samples of broilers in group A showed least TCS value of 26.96±4.9. Group B, C and D showed TCS of 225.99±6.1, 81.3±11.6 and 65.3±17.3 respectively, however group E and F represented TCS of 43.97± 6.4 and 27.61±5.2 respectively. Thus, high degree of DNA damage is observed in the liver sample in group B and C. The sequence for total comet score (TCS) in the liver sample of different groups at the end of the experiment was in the following order B > group C > group D > group E > group F > group A. Thus, the higher total comet score is shown by group B.

Furthermore, kidney samples of broilers in group A showed maximum score of class (0) minimum score of class (4) with TCS of 28.32±6.1. The value of total comet score (TCS) in group B was 235.64±7.9 while that in group C and D was 82.29±5.6 and 66.63±8.6 respectively. Likewise, kidney of chicks from group E and F presented low TCS of 43.98±3.4 and 31.99±4.7 respectively. The sequence for total comet score (TCS)

Table 1s – 1st week

	Comet class and TCS	Group					
		Ochratoxin A 400µg/kg feed					
		A	B	C	D	E	F
Blood	Class 0	92.33± 2.8	59.33± 1.6	71± 2.4	71.66 ± 2.1	75.66± 2.2	91.33± 1.6
	Class 1	3.33 ±1.6	21.66±1.4	18±2.4	18.66±1.7	19±1.2	2.66±2.0
	Class 2	3.66±2.6	8.33±0.8	7.33±0.4	5±0.7	2.33±0.4	3.66±1.4
	Class 3	0.33±0.4	7.66±0.8	1.33±0.4	1.66±0.8	2.33±1.6	0.66±0.4
	Class 4	0.33±0.4	3±0.7	2.33±0.8	1.33±0.4	0.66±0.4	1.66±0.4
	TCS	12.98±9.6	73.3±8.2	45.9±7.6	38.7±7.1	33.3±0.8	18.64±7.6
Liver	Class 0	91.33±1.0	58.66±1.0	69.66±0.4	67.66±4.7	73.66±3.2	90.66±2.2
	Class 1	4.33±0.4	21.66±1.0	18.33±2.8	20.66±1.6	20.33±3.5	2.66±1.0
	Class 2	3.33±1.0	8.66±0.4	8±2.4	4.66±1.0	4.66±1.6	2.33±1.0
	Class 3	0.66±0.4	7.66±0.4	1.66±0.4	1.66±0.8	0.66±0.4	2.66±0.8
	Class 4	0.33±0.40	3.33±0.4	2.33±0.4	1±0	0.66±0.4	1±0.7
	TCS	14.43±7	75.2±4.6	48.6±10.4	38.9±6	34.2±9.5	19.32±8.2
Kidney	Class 0	92.33±1.4	58.66±1.0	63.33±1.0	67.33±0.8	74.33±1.0	91±1.8
	Class 1	2.33±0.4	21.66±0.8	24.3±0.4	24.66±1.6	18.33±0.4	2.33±0.4
	Class 2	4.33±1.7	8±0.7	9±2.1	4.66±1.0	4.66±0.8	4±2.5
	Class 3	0.33±0.4	8.33±0.4	1.66±0.4	1.66±0.4	1.66±0.8	1.66±0.8
	Class 4	0.66±0.4	3.33±0.4	2.33±0.4	1.66±0.8	1±0	1±0.7
	TCS	14.65±6.6	75.9±5	56.6±7.4	45.6±8	36.6±7.6	19.2±10.6

A= pure feed, B= OTA contaminated feed (400µg of OTA/kg of feed), C= OTA contaminated feed (400µg of OTA/kg of feed) + 0.3 % Adsorbent, D= OTA contaminated feed (400µg of OTA/kg of feed) + 0.6 % Adsorbent, E = OTA contaminated feed (400µg of OTA/kg of feed) + 0.9 % Adsorbent, F = OTA contaminated feed (400µg of OTA/kg of feed) + 1.2 % Adsorbent.



in the kidney samples of different groups at the end of the experiment was in order the following order B > group C > group D > group E > group F > group A. Thus, the higher total comet score is shown by group B. While the overall total comet classes scored in blood, liver and kidney of broilers (table 1s to 6s) studied was in order the following order: kidney > liver > blood.

Genotoxicity is a detrimental activity that brings about alterations in cell integrity by affecting its genome (Arbillaga *et al.*, 2007). Several authors and expert groups have concluded that OTA is genotoxic (Sawale *et al.*, 2009). In the present study, groups C, D, E and F were kept on ochratoxin A contaminated feed (400µg OTA/Kg) supplemented with 0.3%, 0.6%, 0.9% and

1.2% (w/w) of the prepared adsorbents respectively. Therefore the rate of total comet score and genotoxicity in blood, kidney and liver samples in group C and D was comparatively less than group B, while group F showed the least value of total comet score followed by group E (among all the adsorbent treated groups). Therefore, group E and F were safe from the genotoxic effects of ochratoxin A which could be correlated to the fact that maximum ochratoxin A has been adsorbed by the adsorbent prepared from coconut shell through the alimentary canal of chicks, which later on passed along with feces. Thus, our findings show maximum efficacy of the prepared adsorbent in ameliorating the toxic effects of ochratoxin A in *Gallus gallusdomesticus*.

Table 2s – 2nd week

	Comet class and TCS	Group					
		Ochratoxin A 400µg/kg feed					
		A	B	C	D	E	F
Blood	Class 0	91.66±1.0	49±0.7	56.6±4.1	69.33±0.4	76.6±0.81	89.6±1.0
	Class 1	1.33±0.4	16±1.2	23.6±1.6	21.33±0.8	19.6±1.0	2.66±0.4
	Class 2	4±0.7	12.6±0.4	9.3±0.8	3±0.7	2.6±0.4	2.33±0.4
	Class 3	0.66±0.4	10.3±1.0	3±0.7	3.33±0.4	0.6±0.4	3±0
	Class 4	2.33±0.4	12±0.7	4±1.2	3±0.7	0.3±0.4	2.33±0.4
	TCS	20.63±4.6	129.3±7.8	67.32±10.1	28.28±4.6	28.28±4.6	25.6±2.8
Liver	Class 0	90.66±1.4	49.3±0.4	61.6±1.4	68.66±1.0	76±1.2	88.66±0.4
	Class 1	3.33±0.4	15±0.7	22.3±1.6	21.33±1.0	19.33±1.6	3±0.7
	Class 2	3±0.7	11.33±0.8	8.3±0.4	3.33±0.8	2.66±0.4	2.66±0.4
	Class 3	0.66±0.8	12±0.7	3.6±0.8	3.66±1.0	1.66±0.4	3±0
	Class 4	2.33±0.4	12.33±0.4	4±0.7	3±0.7	0.33±0.4	2.66±0.4
	TCS	20.63±5.8	122.98±6	65.97±7.6	30.95±5.2	30.95±5.2	27.7±3.1
Kidny	Class 0	89±1.4	55±2.5	60.3±1.0	64±1.2	73.33±1.6	88±1.2
	Class 1	4.66±0.4	17.33±1.0	22±0.7	25±1.4	21.66±1.6	3.33±0.4
	Class 2	2.66±0.4	11.33±0.4	8.6±0.8	3±0.7	3.33±0.4	3±0.7
	Class 3	1.33±0.4	12±0.7	3.6±0.4	4.66±0.4	1.33±0.8	2.66±0.4
	Class 4	2.33±0.4	12.3±0.8	5.3±0.8	3.33±0.4	0.33±0.4	3±0.7
	TCS	23.29±4	125.31±7.1	71.2±6.7	33.3±6.4	33.3±6.4	29.3±5.8

A= pure feed, B= OTA contaminated feed (400µg of OTA/kg of feed), C= OTA contaminated feed (400µg of OTA/kg of feed) + 0.3 % Adsorbent, D= OTA contaminated feed (400µg of OTA/kg of feed) + 0.6 % Adsorbent, E = OTA contaminated feed (400µg of OTA/kg of feed) + 0.9 % Adsorbent, F = OTA contaminated feed (400µg of OTA/kg of feed) + 1.2 % Adsorbent.

Histopathological observations of kidney and liver

Histopathological observations of kidney and liver (6th week) have been presented in (figure 3 and 4: The images of the 1st to the 5th week has not been shown; only observations has been provided). Normal histological patterns with clear glomerular spaces and well conserved tubular structure were shown by the kidneys of chickens in group A throughout the experimental period. Liver also showed normal histological structure in all the six weeks of the experiment. Hepatocytes had centrally placed nuclei with normal cytoplasm and the lobular structure of liver was retained.

The kidneys of the chicken in group B showed a typical histological pattern with slight grade of congestion at some places during the second week of the experiment. The pathological effect of ochratoxin was prominent at the end of the 4th week because some areas of the kidney showed large size nuclei with irreversible condensed DNA in epithelial cells of renal tubules. Urinary spaces were occupied by proliferation of the epithelial cells in glomeruli and marked congestion was noted. Similarly, hepatocytes also showed moderate type of fatty changes. In some areas of the liver section mild type of cellular infiltration was observed around the blood vessels. Moderate degree of swellings and necrosis of hepatocytes was present at most of the



Table 3s – 3rd week

Comet class and TCS		Group					
		Ochratoxin A 400µg/kg feed					
		A	B	C	D	E	F
Blood	Class 0	91±1.2	35.66±1.4	63±0.7	64.6±0.4	72.3±4.2	89.66±0.4
	Class 1	2.66±0.4	14.6±1.0	22.66±0.8	24.33±0.4	25.3±4.6	2.66±0.4
	Class 2	2.33±0.4	13.66±0.8	5±0.7	3±0.7	1.3±0.4	2.33±0.4
	Class 3	2.66±0.4	12.66±0.4	4±0.7	4.33±0.4	0.6±0.4	1.6±0.4
	Class 4	1.33±0.4	23.33±0.8	5.33±0.4	3.66±0.4	0.3±0.4	2.3±0.4
	TCS	20.62±4	173.28±7	65.98±5.9	57.96±4.6	31.29±8.2	21.6±4
Liver	Class 0	90.66±2.2	34.33±0.4	57.66±2.4	64±1.8	70.3±0.4	90.3±1.0
	Class 1	2.33±0.8	14.66±0.4	24±1.4	25.66±1.0	22.66±0.8	3.3±0.4
	Class 2	2.33±0.4	14.66±0.8	5±0.7	3.3±0.8	2.3±0.4	2.3±0.4
	Class 3	3±1.2	13±0.7	4.66±0.4	4.6±0.4	4±0.7	2.3±0.4
	Class 4	1.33±1.0	23.3±0.4	5.33±0.4	4.3±0.4	0.66±0.4	1.6±0.4
	TCS	21.31±9.2	176.3±5.7	69.3±5.6	63.62±5.4	41.9±5.3	21.6±4
Kidney	Class 0	90.66±3.8	32±0.7	61.3±0.4	60.3±0.4	68±0.70	89.6±0.8
	Class 1	2±0.7	15.66±0.4	23.33±0.81	26.6±0.8	23.6±0.4	3.3±0.8
	Class 2	2.66±0.4	15±0.7	5.3±0.4	4±0.7	3.6±0.4	2.66±0.4
	Class 3	2.33±1.7	13.3±0.4	4.3±0.4	4.33±0.4	4.3±0.4	1.33±0.4
	Class 4	2.33±0.4	24±0.7	5.6±0.4	4.66±0.4	0.3±0.4	3±0
	TCS	24.62±8.2	181.6±5.8	69.6±4.4	66.29±5	45.29±4	24.64±2.8

A= pure feed, B= OTA contaminated feed (400µg of OTA/kg of feed), C= OTA contaminated feed (400µg of OTA/kg of feed) + 0.3 % Adsorbent, D= OTA contaminated feed (400µg of OTA/kg of feed) + 0.6 % Adsorbent, E = OTA contaminated feed (400µg of OTA/kg of feed) + 0.9 % Adsorbent, F = OTA contaminated feed (400µg of OTA/kg of feed) + 1.2 % Adsorbent.

Table 4s – 4th week

Comet class and TCS		Group					
		Ochratoxin A 400µg/kg feed					
		A	B	C	D	E	F
Blood	Class 0	90±4.3	28.6±0.8	64.33±0.4	64.33±1.0	68±1.2	91±0.7
	Class 1	2±0.7	16.3±0.4	18±0.7	24.33±0.4	26±1.2	2.3±0.4
	Class 2	1.66±0.8	15.6±0.8	6.33±0.4	4.33±1.7	5±0.7	1.3±0.8
	Class 3	2±0.7	13±0.7	5.66±0.4	3.33±0.4	0.66±0.4	2.6±0.8
	Class 4	2.66±0.4	26.3±0.4	5.66±0.4	3.66±0.4	0.33±0.4	2.6±0.4
	TCS	21.96±6	191.9±5.7	70.28±4.3	57.62±6.6	39.3±5.4	23.3±6
Liver	Class 0	90±0.7	28.6±0.4	63.33±1.4	63.33±1.4	68.3±0.8	93.3±1.0
	Class 1	4±0.7	15.3±0.4	18±0.7	25.33±0.8	25±0.7	1.6±0.8
	Class 2	1.33±0.4	14±0.7	6.66±1.0	4±0.7	5.33±0.4	2.6±0.4
	Class 3	2.33±0.4	13.3±0.4	5±0.7	3.33±0.4	0.66±0.4	3±0.7
	Class 4	2.33±0.4	28.6±0.4	6.33±0.4	4±0.7	0.66±0.4	2±0.7
	TCS	22.97±4.3	197.9±4.6	71.64±6.4	59.32±6.2	40.2±4.3	23.9±6.5
Kidney	Class 0	89.33±0.4	23.6±2.6	61.3±8.3	62.33±1.0	67.33±1.0	89±0.7
	Class 1	3±0.7	18.6±0.81	16.6±1.0	26±1.2	25±0.7	3±0.70
	Class 2	2.33±0.4	16±0.7	7.66±1.0	4±0.7	5.66±0.8	2.3±0.4
	Class 3	2.66±0.4	13±0.7	5.33±0.4	3.33±0.4	1.33±0.8	3.3±0.4
	Class 4	2.66±0.4	30±0.7	6.33±0.4	4.33±0.4	0.66±0.4	2.3±0.4
	TCS	26.28±4.3	209.6±7.1	73.29±5.8	61.31±5.4	42.9±6.3	26.94.3

A= pure feed, B= OTA contaminated feed (400µg of OTA/kg of feed), C= OTA contaminated feed (400µg of OTA/kg of feed) + 0.3 % Adsorbent, D= OTA contaminated feed (400µg of OTA/kg of feed) + 0.6 % Adsorbent, E = OTA contaminated feed (400µg of OTA/kg of feed) + 0.9 % Adsorbent, F = OTA contaminated feed (400µg of OTA/kg of feed) + 1.2 % Adsorbent.

places. During the 6th week of the experimental period, the kidneys as well as the livers showed severe types of pathological effects. Proliferation of the epithelial cells in glomeruli with hyalinization had reached its maximum degree. Extreme congestion was shown by the cells of renal tubules that has narrowed or blocked the lumen

entirely. Similarly, liver also showed severe pathological effects, during the last week of the experimental period that included acute cellular swelling of hepatocytes, congestion, unclear sinusoidal spaces and cell swelling. Individual cell necrosis was present, indicated by pyknosis in the hepatocytes.



Table 5s – 5th week

	Comet class and TCS	Group					
		Ochratoxin A 400µg/kg feed					
		A	B	C	D	E	F
Blood	Class 0	88±0.7	23.66±2.1	60±3.24	64±3.53	69±3.6	87.33±1.4
	Class 1	7.66±0.4	11±0.7	22.33±0.4	27.66±1.4	28.33±2.0	6.33±0.4
	Class 2	2.66±0.4	16.33±1.0	7.33±0.8	4.33±1.0	0.66±0.4	2.33±0.4
	Class 3	1.33±0.4	18.33±1.0	3.33±0.4	3.33±0.4	1.33±0.8	2.66±0.4
	Class 4	0.33±0.4	30.66±1.0	6.66±0.4	3.6±0.4	0.66±0.4	0.66±0.4
	TCS	18.29±4	221.29±9.7	73.62±4.8	60.95±6.2	36.28±6.8	21.62±4
Liver	Class 0	87±0.7	18±3.9	57.66±0.4	60±3.6	69.33±2.9	87±0.7
	Class 1	7.66±0.4	12.33±1.0	25±1.2	28.33±2.9	26.66±1.6	7.66±0.4
	Class 2	2.33±0.4	18±0.7	7±0.7	4±0.7	2.33±0.8	2.33±0.8
	Class 3	2.66±0.4	19.33±0.8	3±0.7	3.33±0.8	0.66±0.4	2.33±0.4
	Class 4	0.33±0.4	30.66±1.0	7.33±0.8	4±0.7	1±0.7	1.66±0.4
	TCS	21.62±4	228.96±8.8	77.32±7.9	62.32±9.5	37.3±7.2	25.95±4.8
Kidney	Class 0	86±1.8	19.66±1.4	55.33±3.8	59.66±1.0	66.33±1.4	86.33±1.0
	Class 1	7.66±0.4	11±0.7	27±1.2	28.33±1.0	31±1.8	6.33±0.4
	Class 2	2.33±0.8	18±0.7	7±0.7	4.33±0.4	0.66±0.8	3±0.7
	Class 3	2.66±0.4	19.66±0.8	4.33±0.4	3.66±0.4	1.66±0.4	2.66±0.4
	Class 4	1.33±0.4	31.66±1.0	7±0.7	4±0	0.33±0.4	1.66±0.4
	TCS	25.62±4.8	232.62±8.5	81.99±6.6	63.97±3	38.6±6.2	26.95±4.6

A= pure feed, B= OTA contaminated feed (400µg of OTA/kg of feed), C= OTA contaminated feed (400µg of OTA/kg of feed) + 0.3 % Adsorbent, D= OTA contaminated feed (400µg of OTA/kg of feed) + 0.6 % Adsorbent, E = OTA contaminated feed (400µg of OTA/kg of feed) + 0.9 % Adsorbent, F = OTA contaminated feed (400µg of OTA/kg of feed) + 1.2 % Adsorbent.

Table 6s – 6th week

	Comet class and TCS	Group					
		Ochratoxin A 400µg/kg feed					
		A	B	C	D	E	F
Blood	Class 0	90±0.7	23±2.1	56.3±2.1	62±2.4	64.3±3.6	90±2.4
	Class 1	3±1.2	11.3±0.8	27±1.8	25±3.0	30.66±2.8	1.66±0.8
	Class 2	1.66±0.4	17±0.7	6±0.7	4.33±0.8	4.33±1.0	2.66±0.4
	Class 3	2.66±0.4	18±0.7	5±0.7	4.66±0.4	0.66±0.4	2.66±0.8
	Class 4	2.66±0.4	30.66±1.4	5.66±0.8	4±0.7	0.33±0.4	3±1.8
	TCS	24.94±4	221.97±9.9	76.64±8.5	63.64±8.6	43.06±7.6	26.96±11.2
Liver	Class 0	89.33±0.4	22.33±0.8	56.33±1.08	61.66±4.9	64.66±1.0	89.33±1.0
	Class 1	2.66±0.4	11.33±0.4	25.66±0.8	26±3.7	29.33±0.4	2.66±0.8
	Class 2	2.33±0.4	16.33±0.4	5.66±2.8	4±1.4	4.33±1.0	1.66±0.8
	Class 3	3±0.7	18±0.7	5±1.2	4.66±0.8	0.66±0.8	2.66±0.4
	Class 4	2.66±0.4	32±0.7	7.33±0.4	4.33±2.1	1±4.8	3.66±0.4
	TCS	26.96±4.9	225.99±6.1	81.3±11.6	65.3±17.3	43.97± 6.4	27.61±5.2
Kidney	Class 0	89±1.8	19±1.2	53.33±2.1	60±3.7	66.33±1.4	88.66±0.8
	Class 1	2.66±0.4	11±0.7	25.66±0.8	27±1.8	25.33±1.4	2±0.7
	Class 2	2.33±0.4	18.33±1.0	5.66±0.4	4±1.2	5.33±0.4	2±0
	Class 3	3±0.7	18.66±0.8	5.33±0.8	4.33±0.4	1.33±0.4	3.33±0.4
	Class 4	3±0.7	33±0.7	7.33±0.4	4.66±0.8	1±0	4±0.7
	TCS	28.32±6.1	235.64±7.9	82.29±5.6	66.63±8.6	43.98±3.4	31.99±4.7

A= pure feed, B= OTA contaminated feed (400µg of OTA/kg of feed), C= OTA contaminated feed (400µg of OTA/kg of feed) + 0.3 % Adsorbent, D= OTA contaminated feed (400µg of OTA/kg of feed) + 0.6 % Adsorbent, E = OTA contaminated feed (400µg of OTA/kg of feed) + 0.9 % Adsorbent, F = OTA contaminated feed (400µg of OTA/kg of feed) + 1.2 % Adsorbent.

The kidney and liver in groups (C, D, E and F) (treated with different concentrations of adsorbent) did not show any violation from the normal histological pattern during the first week of the experimental period. While Mild to moderate degrees of pathological changes were observed during the

third and fourth week of the experimental period in groups C and D of birds that were provided with 0.3 and 0.6% (w/w) of prepared adsorbent mixed with their feed. These pathological changes include slight congestion in the epithelial cells of renal tubules, thickening of the bowman's capsules and larger nuclei

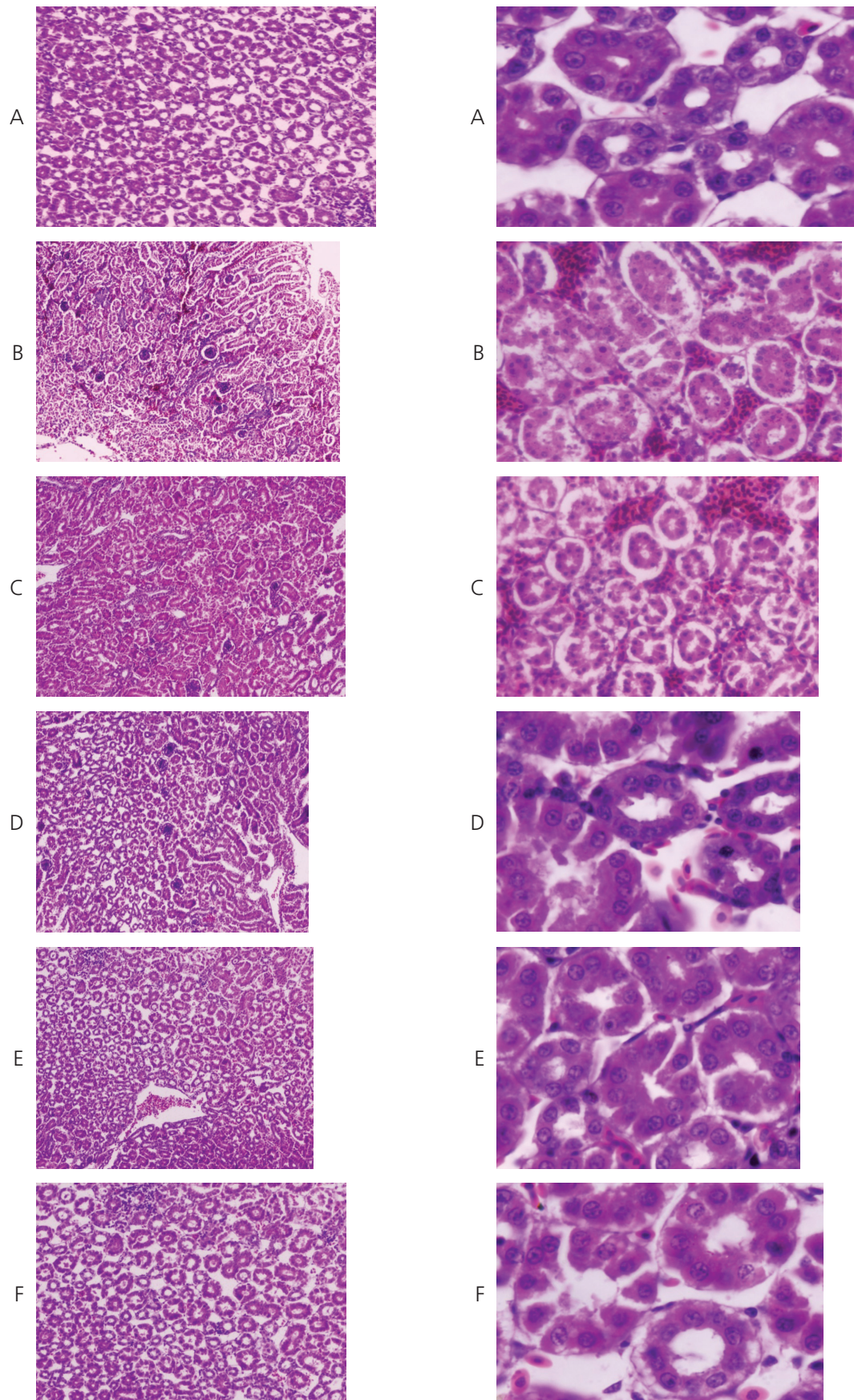


Figure 3 – Photomicrographs showing histopathology of kidney of chickens of 42 days of age in different groups at two different magnifications.

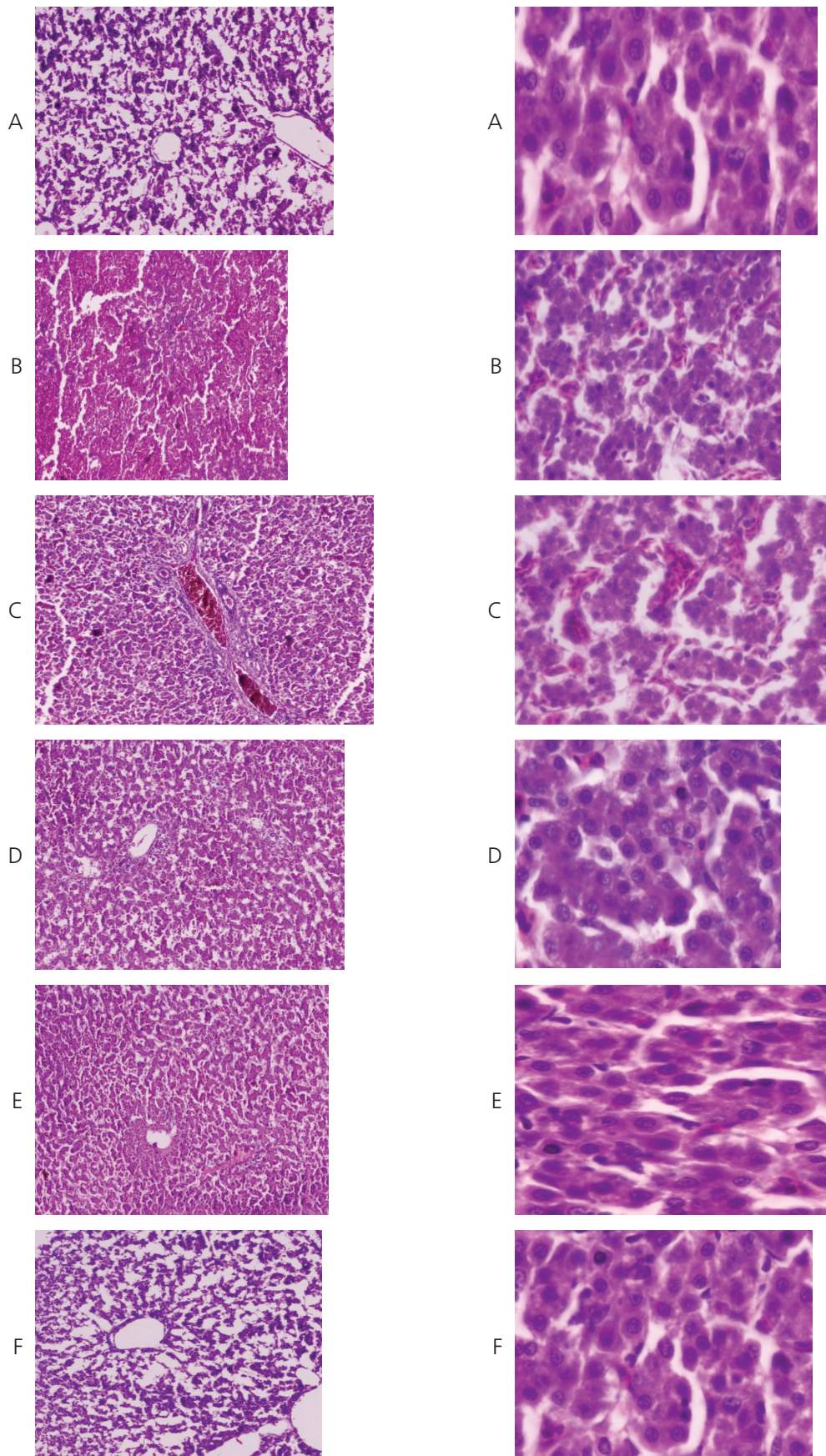


Figure 4 – Photomicrographs showing histopathology of the liver of different groups of chickens of 42 days of age, at two different magnifications.



with irreversible condensed DNA in the epithelial cells of renal tubules. Individual hepatocytic necrosis and hepatocytes swelling of moderate degree was present at some places. Slight degree of cellular diffusion in the interstices around the blood vessels was observed in liver sections. No such pathological changes were observed in the kidney and liver samples in groups E and F of chicken that were kept on feed mixed with 0.9 and 1.2% (w/w) of adsorbent derived from coconut shell. Microscopically, pathological changes observed in the kidneys and liver sections of broiler chicks, exposed to OTA contaminated feed resulted in congestion, necrotic and degenerative changes in the renal tubular epithelial cells. These complications were much prominent in positive controlled group (ochratoxin mixed feed without adsorbent) as compared to other groups. Similar observations regarding OTA induced nephropathy in avian have been reported by Kumar *et al.* (2004).

Gross lesions and morphological alterations

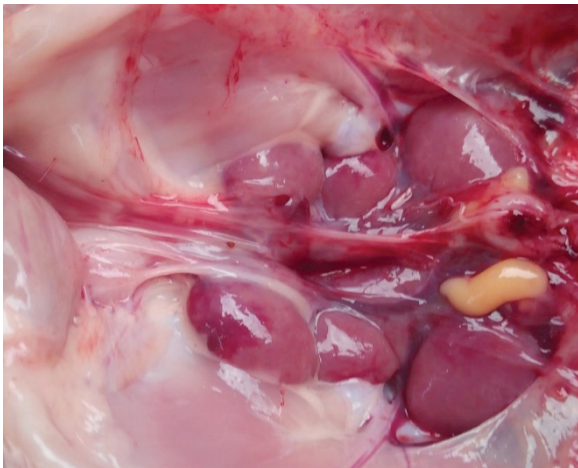
Table (7s) and figure 5 and 6 represent the score of gross lesions in various organs of poultry chicken (1st week to the 6th week). Chickens in group A and

F showed normal morphology of different organs throughout the whole length of the experiment therefore, no gross lesions were observed on kidney, liver and heart. Kidney and liver were normal in size, shape, texture and color. On the other hand, chicks in group B showed gross lesions on kidney and liver. These lesions appeared in the 2nd week on the kidney and in the 3rd week on the liver. The score and severity of gross lesions increased progressively and had reached its maximum during the sixth week of the experiment. Comparatively fewer lesions and morphological alterations were observed by chickens in group C than that in group B. In case in group D and E, minor morphological alterations and very low score of lesions were recorded for kidney. Besides the results in group D and E were found very close to each other. Variation in color, size, texture and frequency of gross lesions in the liver and kidneys of chickens of different groups was subjectively scored by visual examination and has been presented in Table 7s.

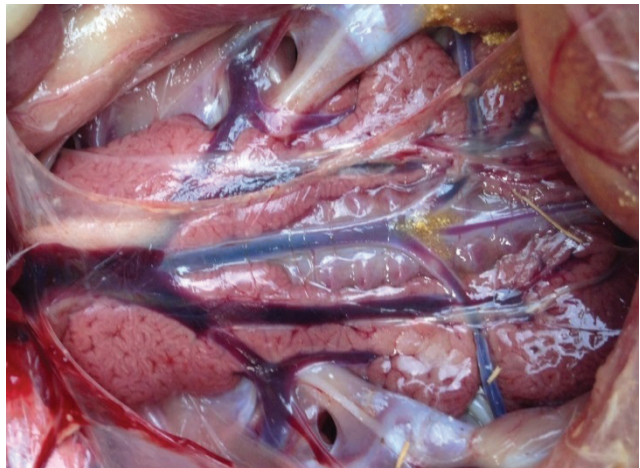
Feeding of OTA to the birds resulted in edema and tubular necrosis particularly in proximal convoluted tubules (Hanif *et al.*, 2006). Ortatatli *et al.* (2005) reported glomerulonephrosis, epithelial cells proliferation and multiple edemas like structures in the

Table 7s – Gross lesions and morphological alterations in different organs of broiler chickens kept on different concentrations of the prepared adsorbent derived from coconut shell.

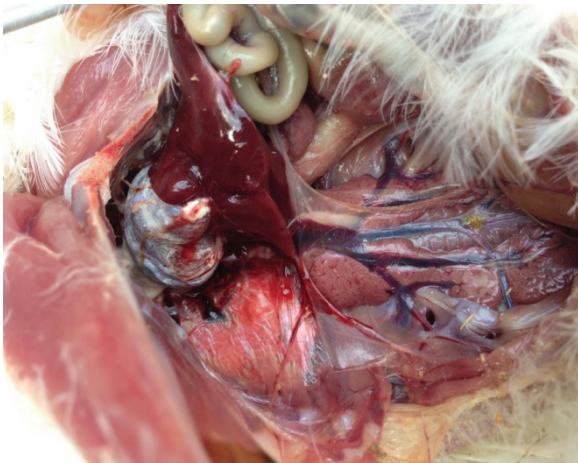
Time period Week (days)	Organs	Maximum possible score	Groups					
			Ochratoxin A 400µg/kg feed					
			A	B	C	D	E	F
First week (7) days	kidney	9	0	0	0	0	0	0
	liver	9	0	0	0	0	0	0
	heart	9	0	0	0	0	0	0
Cumulative score		27	0	0	0	0	0	0
second week (14) days	kidney	9	0	3	0	1	0	0
	liver	9	0	0	0	0	0	0
	heart	9	0	0	0	0	0	0
Cumulative score		27	0	3	0	1	0	0
third week (21) days	kidney	9	0	5	1	0	0	0
	liver	9	0	3	0	0	0	0
	heart	9	0	0	0	0	0	0
Cumulative score		27	0	8	1	0	0	0
Forth week (28) days	kidney	9	0	7	3	1	0	0
	liver	9	0	3	1	0	0	0
	heart	9	0	0	0	0	0	0
Cumulative score		27	0	10	4	1	0	0
Fifth week (35) days	kidney	9	0	8	3	1	0	0
	liver	9	0	4	1	0	0	0
	heart	9	0	0	0	0	0	0
Cumulative score		27	0	12	4	1	0	0
Sixth week (42) days	kidney	9	0	8	3	1	1	0
	liver	9	0	5	2	0	0	0
	heart	9	0	0	0	0	0	0
Cumulative score		27	0	13	5	1	1	0



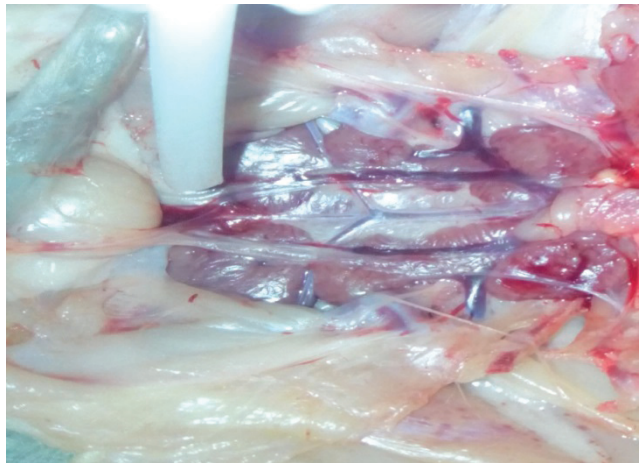
A



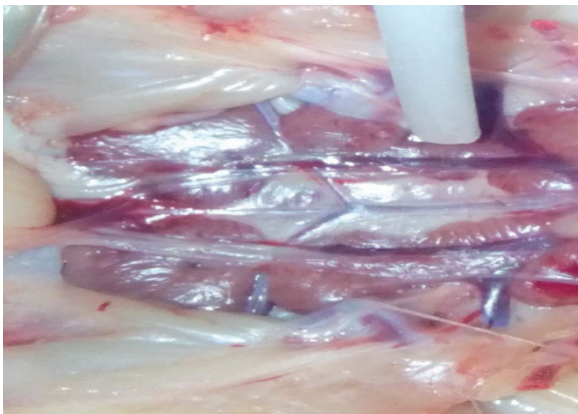
B



C



D



E

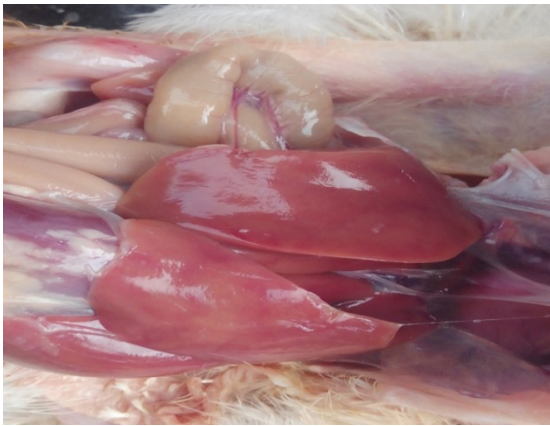


F

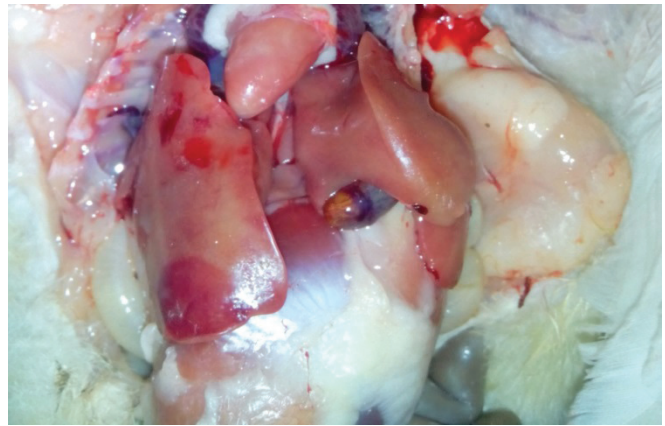
Figure 5 – Photomicrographs showing kidney lesions in different groups.

kidneys parenchyma cells in chickens fed on OTA contaminated diet. The lesions observed in the liver and kidneys of chicks in group "B" sacrificed at the end of the experiment week, showed enlarged kidneys with prominent lesions. Also, pale friable liver with petechiae on the surface were also observed. Swollen

kidneys and liver with pinpoint hemorrhages in laying hens and in broiler chicks have been described by several researchers (Kumar *et al.*, 2004; Politis *et al.*, 2005). Gross lesions on different organs in broiler chicks during ochratoxicosis have also been reported (Arbillaga *et al.*, 2007).



A



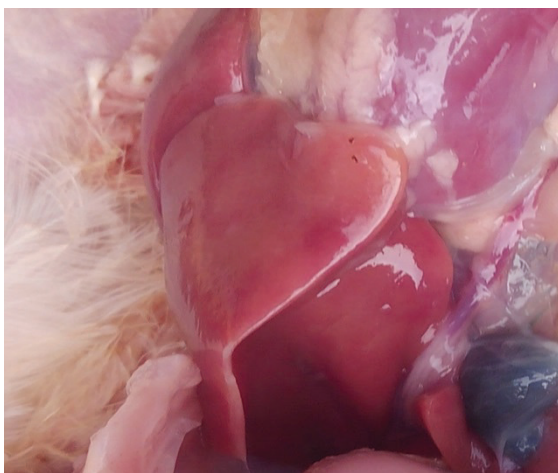
B



C



D



E



F

Figure 6 – Photomicrographs showing liver lesions in different groups.

Enlargement of different organs

Table 8s indicated relative enlargement of different organs of chicks during six weeks of the experiment (1st week to the 6th week). Normal size and appearance of visceral organs were shown by chickens in group A,

E and F during the entire experimental duration. Under keen observation, the kidneys in group B showed slight enlargement during the 2nd week, whereas the liver remained normal. Minor enlargement of the liver was shown by birds in group B during the third week of the experiment. The degree of enlargement of visceral



Table 8s – Enlargement of different organs of broiler chickens kept on different concentrations of the prepared adsorbent derived from coconut shell.

Time period	Organs	Maximum possible score	Groups					
			Ochratoxin A 400µg/kg feed					
Week(days)			A	B	C	D	E	F
First week (7) days	kidney	9	0	0	0	0	0	0
	liver	9	0	0	0	0	0	0
	heart	9	0	0	0	0	0	0
Cumulative score		27	0	0	0	0	0	0
second week (14) days	kidney	9	0	2	0	0	0	0
	liver	9	0	0	0	0	0	0
	heart	9	0	0	0	0	0	0
Cumulative score		27	0	2	0	0	0	0
third week (21) days	kidney	9	0	3	2	1	0	0
	liver	9	0	1	0	0	0	0
	heart	9	0	0	0	0	0	0
Cumulative score		27	0	4	2	1	0	0
Forth week (28) days	kidney	9	0	6	3	0	0	0
	liver	9	0	2	1	0	0	0
	heart	9	0	0	0	0	0	0
Cumulative score		27	0	8	4	0	0	0
Fifth week (35) days	kidney	9	0	6	3	1	0	0
	liver	9	0	3	1	0	0	0
	heart	9	0	0	0	0	0	0
Cumulative score		27	0	9	4	1	0	0
Sixth week (42) days	kidney	9	0	7	3	1	0	0
	liver	9	0	2	1	0	0	0
	heart	9	0	0	0	0	0	0
Cumulative score		27	0	9	4	1	0	0

organs (kidney and liver) in group B increased with the increase in exposure duration of chicks to ochratoxin A contaminated feed. The kidneys in group C (0.3% w/w) and D (0.6% w/w) showed very low degree of enlargement when compared to group B. However, kidney, liver and heart in group E and F showed normal appearance and no enlargement were observed at all during the experiment. The sequence for overall enlargement of kidney and liver was in the following order: group B > group C > group D > throughout the experimental weeks, while group A, E and F have shown normal size of these organs.

Kidney enlargements and moderate liver enlargement in intoxicated broiler chicks have also been documented by Verma *et al.* (2004) as well. It was concluded that the addition of different levels of adsorbent in ochratoxin A contaminated feed ameliorated the hazardous effects of ochratoxin A.

Relative body weight

Table 9s represented relative body weights of chicks kept on different concentrations of the prepared adsorbent derived from coconut shell (1st week to the 6th week). There was no significant difference in

the body weight of chickens in group A and D. Birds in group A and C presented a relative significant difference at the end of the 3rd, 4th, 5th and 6th weeks. Similarly, group C, D, E and F also exhibited higher significant differences when compared to group B. The maximum body weights were observed in group F followed by group E and A.

Early researchers have observed reduction in body weight gain of chicks when exposed to dietary ochratoxin A contaminated feed (Peckham *et al.*, 1971; Kumar *et al.*, 2004). Our study showed that body weight of chicks in group B decreased throughout the course of the study. The food conversion ratio (FCR) in group B was also impaired, whereas those groups receiving ochratoxin A contaminated feed along with different levels of adsorbent showed increase in body weight and normal food conversion ratio (FCR). Reduction in weight gain of chicks in group B may be due to less attraction toward feed. Hypoproteinaemia and low level of somatotrophic hormones, result from hepatotoxicity which leads to low feed intake and reduced weight gain in chickens exposed to the experimental ochratoxicosis (Elaroussi *et al.*, 2006). These results are also in line with the



Table 9s – Relative body weights (g) of broiler chickens kept on different concentrations of the prepared adsorbent derived from coconut shell.

Time period	Groups					
	Ochratoxin A 400µg/kg feed					
Week (days)	A	B	C	D	E	F
1 (07)	140.49 ^b ±0.6925	140.85 ^b ±0.5691	139.93 ^b ±0.7110	140.57 ^b ±0.5577	141.22 ^b ±0.6352	142.57 ^a ±0.6240
2 (14)	475.78 ^a ±3.6336	360.07 ^e ±2.4622	396.28 ^d ±3.6530	443.89 ^c ±3.7919	460.80 ^b ±3.9359	471.55 ^a ±3.8013
3 (21)	920.66 ^a ±11.621	579.85 ^c ±12.822	626.70 ^b ±11.269	913.18 ^a ±13.015	931.62 ^a ±12.117	906.25 ^a ±12.019
4 (28)	1448.2 ^a ±16.681	939.93 ^c ±9.7359	1060.4 ^b ±7.7620	1445.0 ^a ±17.657	1461.8 ^a ±17.684	1439.7 ^a ±16.866
5 (35)	1854.0 ^{ab} ±13.116	1269.4 ^d ±12.282	1382.3 ^c ±15.297	1823.9 ^{ab} ±16.098	1863.4 ^a ±11.899	1835.0 ^{ab} ±10.917
6 (42)	2092.6 ^a ±20.390	1360.9 ^c ±15.697	1475.2 ^b ±11.534	2065.2 ^a ±30.468	2093.1 ^a ±21.989	2117.6 ^a ±20.586

present study suggesting that reduced weight gain and low feed ingestion was due to hampered protein synthesis by OTA which acts as competitive inhibitor of phenylalanine, preventing the attachment of this specific amino acid to "Phenylalanine transfer RNA synthetase" enzyme (Kubina *et al.*, 1990).

Relative kidney weight

Relative kidney weights of broiler chicks (1st week to the 6th week) given different levels of adsorbent have been shown in Table (10s). Chicks in group B showed significantly higher values than group A throughout the experimental period. No significant difference was found in the values of kidneys of broiler chicks in group A, D, E and F during the 1st week. No significant

difference was observed in the values of relative weight of kidneys in group A, E and F chicks at the end of the 2nd, 3rd and 6th week. Higher significant difference was observed in the values in group B when compared with the values in group D, E and F fed with different level of the prepared adsorbent. In the present study chicks in group B fed on OTA contaminated feed only (positive control) exhibited increased weight of kidneys. Similar alterations in kidneys have been reported in different avian species (Khadem *et al.*, 2012). The increase in weight of kidney and liver might be associated with the route of elimination of OTA. Toxic effects of OTA upon these organs might have impaired their efficiency of eliminating toxin and resulted in accumulation in these organs.

Table 10s – Relative kidney weights (g) of broiler chickens kept on different concentrations of the prepared adsorbent derived from coconut shell.

Time period	Groups					
	Ochratoxin A 400µg/kg feed					
Week (days)	A	B	C	D	E	F
1 (07)	0.6567 ^b ±0.0176	0.6933 ^a ±0.0167	0.6633 ^b ±0.0279	0.6567 ^b ±0.0120	0.6467 ^b ±0.0267	0.6400 ^b ±0.0153
2 (14)	0.7233 ^b ±0.0176	0.7833 ^a ±0.0176	0.7600 ^{ab} ±0.0208	0.7467 ^{ab} ±0.0186	0.7233 ^b ±0.0120	0.7067 ^b ±0.0260
3 (21)	0.6533 ^c ±0.0233	0.8600 ^a ±0.0252	0.7933 ^b ±0.0120	0.6967 ^c ±0.0318	0.6767 ^c ±0.0120	0.6900 ^c ±0.0551
4 (28)	0.7200 ^d ±0.0208	0.9133 ^a ±0.0185	0.8533 ^b ±0.0145	0.7900 ^c ±0.0115	0.7433 ^{cd} ±0.0416	0.7333 ^d ±0.0219
5 (35)	0.7333 ^d ±0.0186	0.9900 ^a ±0.0208	0.8633 ^b ±0.0328	0.8100 ^{bc} ±0.0115	0.7700 ^{cd} ±0.0115	0.7733 ^{cd} ±0.0176
6 (42)	0.7167 ^d ±0.0203	1.0267 ^a ±0.0338	0.9333 ^b ±0.0145	0.8400 ^c ±0.0200	0.7400 ^d ±0.0208	0.7500 ^d ±0.0200

Relative liver weight

Table (11s) has shown the relative liver weights of broiler chicks given different levels of adsorbent (1st week to the 6th week). The mean value of liver weight of broiler chicks in group B was found significantly higher than group A throughout the experimental period. No significant difference was found in the values of the liver of broiler chicks in group A, D, E and F at the end of the fourth week. Relative weights of the liver of broiler chicks were not significantly different in groups A, E and F when observed at the end of the 2nd, 4th and 5th week. Higher significant difference was observed in the values in group B when compared with

the values in group D, E and F fed with different level of the prepared adsorbent.

In the present study increase in relative weight of liver in chicks in group B may be due to deposition of fats in the liver formerly described by several researchers (Khadem *et al.*, 2012). No pathological signs were observed in chickens in groups D, E and F fed on ochratoxin A contaminated feed supplemented with different levels of adsorbent and the toxic effects of ochratoxin A were diminished in poultry birds. The main reason behind that is that the adsorbent has showed strong adsorption capacity for ochratoxin A that occurred from the gastrointestinal tract of broilers and thus these chicks were safe from the detrimental



Table 11s – Relative weight (g) of the liver of broiler chickens kept on different concentrations of the prepared adsorbent derived coconut shell.

Time period	Groups					
	Ochratoxin A 400µg/kg feed					
Week (days)	A	B	C	D	E	F
1 (07)	3.7567 ^c ±0.0437	4.0767 ^a ±0.0448	3.9567 ^{ab} ±0.0788	3.8367 ^{bc} ±0.0644	3.7700 ^c ±0.0819	3.7967 ^{bc} ±0.0719
2 (14)	3.9767 ^c ±0.0751	4.5033 ^a ±0.0837	4.2433 ^{ab} ±0.1040	4.1100 ^{bc} ±0.0529	3.9533 ^c ±0.1267	3.8800 ^c ±0.0361
3 (21)	4.0567 ^{bc} ±0.0769	4.3867 ^{ab} ±0.1450	4.4567 ^a ±0.1963	4.1100 ^{abc} ±0.0529	3.9933 ^c ±0.0696	3.8833 ^c ±0.054
4 (28)	4.0500 ^b ±0.0346	5.3000 ^a ±0.2237	4.9467 ^a ±0.0857	4.3267 ^b ±0.1539	4.0033 ^b ±0.0590	4.0600 ^b ±0.0635
5 (35)	3.8667 ^d ±0.0775	6.4367 ^a ±0.2314	5.5167 ^b ±0.2367	4.6400 ^c ±0.1415	3.8067 ^d ±0.0617	3.8867 ^d ±0.1126
6 (42)	3.8833 ^a ±0.0706	6.9233 ^b ±0.0996	6.2533 ^c ±0.2073	4.9900 ^d ±0.0917	3.8633 ^d ±0.0353	3.8700 ^d ±0.0458

effects of the mycotoxin (Pasha *et al.*, 2007; Singh *et al.*, 1990).

Relative spleen weight

Relative spleen weights of broiler chickens given different level of adsorbent have been shown in Table (12s). The mean value of spleen weight of broiler chicks in group B was found significantly higher than group A throughout the experimental period. No significant difference was found in the values of spleen of broiler chicks in group A, C, D, E and F at the end of the 2nd and 3rd week. Higher significant difference was observed in the values in group B when compared with

the values in group C, D, E and F fed with different levels of the prepared adsorbents. No alteration was found in relative weight of spleen in chicks in group E and F throughout the whole experimental duration because these groups were provided with adsorbent at concentration of 0.9% and 1.2% (w/w) respectively which indicated maximum effectiveness of the prepared adsorbents. These results were in accordance with the work of other authors, in which they successfully used adsorbents to ameliorate the toxic effects of ochratoxin A in different organs of chicks including spleen and Bursa of fabricius (Singh *et al.*, 1990; Campbell *et al.*, 1983; Pasha *et al.*, 2007; Khadem *et al.*, 2012).

Table 12s – Relative weight (g) of spleen of broiler chickens kept on different concentrations of the prepared adsorbent derived from coconut shell.

Time period	Groups					
	Ochratoxin A 400µg/kg feed					
Week (days)	A	B	C	D	E	F
1 (07)	0.0567 ^c ±0.0002	0.1233 ^a ±0.0004	0.0833 ^b ±0.0001	0.0700 ^{bc} ±0.0001	0.0633 ^c ±0.0002	0.0667 ^{bc} ±0.0002
2 (14)	0.0567 ^c ±0.0002	0.1367 ^a ±0.0145	0.1067 ^b ±0.0120	0.0767 ^c ±0.0002	0.0733 ^c ±0.0003	0.0533 ^c ±0.0003
3 (21)	0.0667 ^c ±0.0003	0.1133 ^a ±0.0003	0.0933 ^b ±0.0002	0.0767 ^c ±0.0004	0.0700 ^c ±0.0000	0.0700 ^c ±0.0003
4 (28)	0.0767 ^c ±0.0002	0.1500 ^a ±0.0115	0.1133 ^b ±0.0003	0.0933 ^{bc} ±0.0003	0.0733 ^c ±0.0002	0.0800 ^c ±0.0000
5 (35)	0.0533 ^c ±0.0003	0.1067 ^a ±0.0145	0.0833 ^{ab} ±0.0002	0.0767 ^{bc} ±0.0003	0.0633 ^{bc} ±0.0004	0.0733 ^{bc} ±0.0003
6 (42)	0.0633 ^c ±0.0003	0.1167 ^a ±0.0120	0.0967 ^{ab} ±0.0004	0.0833 ^{bc} ±0.0003	0.0700 ^c ±0.0000	0.0667 ^c ±0.0003

Relative weight of bursa of fabricius

Relative weights of bursa of fabricius of broiler chickens (1st week to the 6th week) given different levels of adsorbent have been shown in Table (13s). The mean values of weight of bursa of fabricius in

group B were found significantly lower than group A throughout the experimental period. No significant difference was found in the values of bursa of fabricius of broiler chicks in group A, E and F at the end of the 1st and 4th weeks. Higher significant difference was observed in the values in group B when compared with

Table 13s – Relative weight (g) of bursa of fabricius of broiler chickens kept on different concentrations of the prepared adsorbent derived from coconut shell.

Time period	Groups					
	Ochratoxin A 400µg/kg feed					
Week (days)	A	B	C	D	E	F
1 (07)	0.1533 ^a ±0.0125	0.1000 ^b ±0.0120	0.1300 ^{ab} ±0.0115	0.1367 ^{ab} ±0.0145	0.1500 ^a ±0.0153	0.1567 ^a ±0.0145
2 (14)	0.1300 ^a ±0.0115	0.0833 ^c ±0.0003	0.1067 ^{bc} ±0.0120	0.1133 ^{abc} ±0.0004	0.1433 ^{ab} ±0.0120	0.1367 ^{ab} ±0.0145
3 (21)	0.1100 ^{abc} ±0.0115	0.0767 ^d ±0.0004	0.0900 ^{cd} ±0.0003	0.1033 ^{bc} ±0.0002	0.1267 ^a ±0.0004	0.1133 ^{ab} ±0.0003
4 (28)	0.1600 ^a ±0.0153	0.1100 ^b ±0.0115	0.1133 ^b ±0.0003	0.1267 ^{ab} ±0.0145	0.1633 ^a ±0.0120	0.1633 ^a ±0.0167
5 (35)	0.1667 ^a ±0.0003	0.1200 ^c ±0.0004	0.1367 ^{bc} ±0.0002	0.1467 ^{ab} ±0.0003	0.1500 ^{ab} ±0.0100	0.1667 ^a ±0.0002
6 (42)	0.0933 ^{bc} ±0.0003	0.0633 ^d ±0.0003	0.0800 ^c ±0.0000	0.0867 ^c ±0.0002	0.1067 ^{ab} ±0.0004	0.1167 ^a ±0.0003



the values in group C, D, E and F fed on different levels of the prepared adsorbent, however close resemblance was shown by the values in group D, E and F with that in group A.

The relative weight of bursa of fabricius also showed exposure duration related decreased, upon feeding OTA contaminated feed. In the present study chicks in group B (fed with ochratoxin contaminated feed only) exhibited decrease in weight of bursa of fabricius. A decrease in the weight of bursa of fabricius has also been quoted by several researchers (Marquize *et al.*, 1995). Campbell *et al.* (1983) reported a decrease in the weight of bursa of fabricius in broiler chicks by concurrent feeding of OTA and aflatoxin contaminated feed. Many researchers reported a decrease in the relative weights of thymus and bursa of fabricius using different dietary levels of OTA (O'Brien *et al.*, 2005). The findings of the present study also supported the previous reports. According to Raju *et al.* (2002), the decrease in weight of bursa of fabricius is due to the suppression of the immune system when fed on ochratoxin A contaminated diet.

Serum biochemistry

Serum alanine transferase (ALT) and alkaline phosphatase (ALP)

The biochemical analysis of blood serum of broiler chickens for ALT and ALP concentration (1st week to the 6th week) is shown in (Table 14s and 15s). ALT and ALP concentrations in groups B and C were significantly

higher than D, E and F throughout the experimental period. Relative ALT and ALP concentration of broiler chicks was noted non-significant in groups A, E and F at the end of the 3rd, 4th, 5th and 6th weeks of the experiment, however lower values were recorded in group E and F. Higher significant difference was observed in the values in group B when compared with the values in group C, D, E and F provided with diet supplemented by 400 ppb of OTA along with 0.3%, 0.6%, 0.9% and 1.2% (w/w) of the prepared adsorbents respectively.

Severe toxicological effects were reported in the serum biochemical parameters of the chicks fed OTA contaminated feed (Verma *et al.*, 2003). In the present study positive control group (fed with ochratoxin contaminated feed only) has shown the highest level of serum of ALT and ALP concentration. However negative control group and adsorbent treated groups have shown comparatively low concentrations. Our results showed that the ALT and ALP levels of chicks in group A, E and F were not significantly different from each other. This indicated that chicks in group E and F have normal ALT and ALP concentrations as compared to negative control group (A). Thus our findings revealed that 0.9% of the prepared nano-composites (adsorbent) are enough to subside the toxic effects of ochratoxin A in *Gallus gallusdomesticus*. Such outcomes have also been reported by several researchers (Zahoor & Khan 2014; Koynarski *et al.*, 2007).

Table 14s – Serum ALT concentration in broiler chickens kept on different levels of the prepared adsorbent derived from coconut shell.

Time Period	Group					
	Ochratoxin A 400µg/kg feed					
Week (days)	A	B	C	D	E	F
1 (07)	10.750 ^b ±0.2343	11.717 ^a ±0.1683	11.180 ^b ±0.0702	11.113 ^b ±0.1184	11.093 ^b ±0.0644	10.877 ^b ±0.1880
2 (14)	12.287 ^d ±0.0895	14.680 ^a ±0.1966	13.947 ^b ±0.3671	12.887 ^c ±0.075	12.51 ^{cd} ±0.067	12.35 ^{cd} ±0.177
3 (21)	14.387 ^c ±0.1707	19.687 ^a ±0.461	17.360 ^b ±0.424	15.097 ^c ±0.1122	14.493 ^c ±0.1419	14.393 ^c ±0.167
4 (28)	12.453 ^d ±0.219	21.353 ^a ±0.469	17.93 ^b ±0.6533	15.513 ^c ±0.18	13.127 ^d ±0.0694	12.94 ^d ±0.071
5 (35)	13.550 ^d ±0.189	36.213 ^a ±0.619	21.307 ^b ±0.482	16.537 ^c ±0.403	13.94 ^d ±0.221	13.887 ^d ±0.233
6 (42)	12.530 ^d ±0.2163	48.857 ^a ±0.479	25.237 ^b ±0.466	17.567 ^c ±0.257	13.387 ^d ±0.0437	13.277 ^d ±0.0788

Table 15s – Serum alkaline phosphatase (ALP) concentrations (U/L) in broiler chickens kept on different levels of the prepared adsorbent derived from coconut shell.

Time Period	Group					
	Ochratoxin A 400µg/kg feed					
Week (days)	B	C	D	E	F	
1 (07)	627.59 ^b ±8.171	650.33 ^a ±2.905	639.33 ^{ab} ±1.201	636.00 ^{ab} ±2.645	629.00 ^b ±4.163	628.33 ^b ±6.765
2 (14)	646.50 ^b ±2.762	670.00 ^a ±1.15	655.33 ^b ±5.174	648.33 ^b ±4.409	647.67 ^b ±3.38	646.67 ^b ±4.841
3 (21)	595.33 ^d ±2.8954	690.67 ^a ±3.480	671.00 ^b ±6.658	630.33 ^c ±5.487	599.00 ^d ±3.055	597.33 ^d ±2.33
4 (28)	619.07 ^d ±4.217	858.33 ^a ±10.13	805.00 ^b ±5.033	726.33 ^c ±6.333	619.33 ^d ±1.855	618.67 ^d ±0.8819
5 (35)	665.46 ^d ±4.11	961.67 ^a ±4.409	879.00 ^b ± 0.57	782.00 ^c ±7.00	666.33 ^d ±3.756	666.33 ^d ±2.962
6 (42)	643.25 ^d ±5.01	1124.3 ^a ±8.089	990.67 ^b ±5.36	799.00 ^c ±6.928	701.33 ^d ±6.64	662.33 ^d ±5.044



Serum creatinine and urea

The biochemical analysis of blood serum of broiler chickens for creatinine and urea concentration (1st week to the 6th week) is shown in (Table 16s and 17s). Mean value of creatinine and urea concentration in group E and F were significantly lower than that in group B and C throughout the experimental period. No significant differences were found in the values of creatinine concentration of broiler chicks in group A, C, D, E and F at the end of the 1st week of the experiment. Relative creatinine and urea concentrations of broiler chicks were noted non-significant in groups A, E and F at the end of the 3rd, 4th, 5th and 6th week of the experiment.

In the present study, creatinine and urea concentration in group B was significantly higher than

group A (negative control group) and adsorbent treated groups (particularly D, E and F) at 42 days of experiment. Our findings of increased creatinine and urea values coincide with the results previously reported in White Leghorn and broilers (Kumaret *al.*, 2004). Significantly increased levels of serum creatinine and urea in the OTA treated group in addition to histological changes also confirm impaired renal function and primary site for OTA action. Our results showed that the addition of the adsorbent to ochratoxin A contaminated feed strongly ameliorated the toxic effects of ochratoxin A in *Gallus gallusdomesticus*. Similar findings have been reported by various researchers. They demonstrated that different adsorbents have the capability to reduce the toxic effect in layer and in chicks when offered ochratoxin A contaminated feed broilers (Kumar *et al.*, 2004).

Table 16s – Serum creatinine concentrations (mg/dl) in broiler chickens kept on different levels of the prepared adsorbent derived from coconut shell.

Time Period	Group					
	Ochratoxin A 400µg/kg feed					
Week (days)	A	B	C	D	E	F
1 (07)	0.4687 ^b ± 0.0527	0.6333 ^a ± 0.0285	0.5973 ^{ab} ± 0.02	0.4797 ^b ± 0.01	0.4723 ^b ± 0.0521	0.4613 ^b ± 0.0551
2 (14)	0.4747 ^b ± 0.0542	0.7333 ^a ± 0.0260	0.7033 ^a ± 0.0145	0.5060 ^b ± 0.0465	0.4770 ^b ± 0.0533	0.4667 ^b ± 0.0354
3 (21)	0.4680 ^b ± 0.0367	0.8453 ^a ± 0.0230	0.7983 ^a ± 0.02	0.4960 ^b ± 0.0202	0.4723 ^b ± 0.0372	0.4657 ^b ± 0.0394
4 (28)	0.4663 ^c ± 0.0388	0.8910 ^a ± 0.0260	0.8080 ^b ± 0.01	0.4933 ^c ± 0.0169	0.4750 ^c ± 0.0378	0.4753 ^c ± 0.0125
5 (35)	0.4663 ^c ± 0.0388	0.8910 ^a ± 0.0260	0.8080 ^b ± 0.02	0.4933 ^c ± 0.0169	0.4750 ^c ± 0.0378	0.4753 ^c ± 0.0125
6 (42)	0.4950 ^c ± 0.02	1.1900 ^a ± 0.1130	0.9963 ^b ± 0.01	0.5203 ^c ± 0.0170	0.5013 ^c ± 0.01	0.4973 ^c ± 0.01

Table 17s – Serum urea concentrations (mg/dl) in broiler chickens kept on different levels of the prepared adsorbent derived from coconut shell.

Time Period	Group					
	Ochratoxin A 400µg/kg feed					
Week (days)	A	B	C	D	E	F
1 (07)	11.343 ^c ± 0.2742	13.153 ^a ± 0.0821	12.503 ^b ± 0.2564	12.093 ^b ± 0.0521	12.100 ^b ± 0.0416	11.357 ^c ± 0.1670
2 (14)	11.657 ^e ± 0.1934	15.180 ^a ± 0.0361	14.087 ^b ± 0.0448	13.107 ^c ± 0.0581	12.137 ^d ± 0.0328	11.663 ^d ± 0.1934
3 (21)	11.660 ^d ± 0.1858	17.157 ^a ± 0.0233	16.180 ^b ± 0.0907	14.653 ^c ± 0.1934	12.063 ^d ± 0.0437	11.667 ^d ± 0.1805
4 (28)	11.727 ^c ± 0.0982	19.183 ^a ± 0.0584	18.810 ^a ± 0.1850	15.447 ^b ± 0.2788	12.143 ^c ± 0.0517	11.753 ^c ± 0.1186
5 (35)	11.750 ^c ± 0.2212	21.170 ^a ± 0.0917	20.100 ^b ± 0.1002	16.137 ^c ± 0.0684	12.160 ^d ± 0.1069	11.773 ^d ± 0.1129
6 (42)	11.713 ^d ± 0.1053	23.433 ^a ± 0.2603	21.207 ^b ± 0.1087	17.140 ^c ± 0.1242	12.153 ^d ± 0.1444	11.777 ^d ± 0.09

Serum albumen, total proteins and globulin

Relative serum albumen, total proteins and globulin concentrations of broiler chicks (1st week to the 6th week) given different levels of adsorbent is shown in tables (18s and 19s). No significant difference was found in the values of serum albumen, total proteins and globulin of broiler chicks in groups A, E and F at the end of the experiment. However the values in group C for the mentioned parameters were significantly different when compared to group A, E and F throughout the experiment. Higher significant difference was observed in the values in group B when

compared with the values in groups D, E and F fed with different levels of adsorbent prepared from coconut shell. Chicks in group B (positive control) showed the lowest levels of serum albumen, total proteins and globulin.

In our study low values of serum albumen, total proteins and globulin in chicken fed with ochratoxin A contaminated feed may be due to the declined synthesis in the the liver suffering from hepatic toxicity (Zahoor & Khan 2015). In serum biochemical parameters, globulin level was significantly lower in chicks in group B (fed with toxin contaminated feed only) than that in group A fed with basal feed (negative control).



Table 18s – Serum albumen concentrations (g/dl) in broiler chickens kept on different levels of the prepared adsorbent derived from coconut shell.

Time Period	Group					
	Ochratoxin A 400µg/kg feed					
Week (days)	A	B	C	D	E	F
1 (07)	2.21 ^a ±0.0153	1.96 ^d ±0.005	2.0133 ^c ±0.008	2.0733 ^b ±0.012	2.19 ^a ±0.0176	2.20 ^a ±0.0289
2 (14)	2.34 ^a ±0.0145	1.95 ^d ±0.0145	2.12 ^c ±0.008	2.19 ^b ±0.0115	2.36 ^a ±0.0203	2.38 ^a ±0.0208
3 (21)	2.39 ^a ±0.0115	2.05 ^d ±0.0115	2.17 ^c ±0.0145	2.21 ^b ±0.003	2.36 ^a ±0.003	2.37 ^a ±0.0219
4 (28)	2.22 ^a ±0.0145	1.95 ^d ±0.003	2.04 ^c ±0.024	2.10 ^b ±0.0120	2.20 ^a ±0.0167	2.21 ^a ±0.0231
5 (35)	2.40 ^a ±0.0120	2.10 ^c ±0.003	2.15 ^c ±0.0115	2.2867 ^b ±0.003	2.36 ^{ab} ± 0.0557	2.40 ^a ±0.003
6 (42)	3.56 ^a ±0.1255	2.95 ^d ±0.0837	3.18 ^c ±0.0351	3.32 ^b ±0.0536	3.12 ^a ±0.432	3.51 ^a ±0.1273

Table 19s – Serum total protein concentration (g/dl) in broiler chickens kept on different levels of the prepared adsorbent derived from coconut shell.

Time Period	Group					
	Ochratoxin A 400µg/kg feed					
Week (days)	A	B	C	D	E	F
1 (07)	3.42 ^a ±0.017	3.02 ^d ±0.020	3.06 ^d ±0.027	3.19 ^c ±0.09	3.32 ^b ±0.01	3.39 ^{ab} ±0.01
2 (14)	3.55 ^a ±0.01	3.04 ^d ±0.03	3.26 ^c ±0.01	3.37 ^b ±0.021	3.54 ^a ±0.02	3.59 ^a ±0.01
3 (21)	3.56 ^a ±0.01	2.93 ^d ±0.017	3.19 ^c ±0.25	3.36 ^b ±0.02	3.53 ^a ±0.03	3.56 ^a ±0.02
4 (28)	3.45 ^a ±0.015	2.68 ^c ±0.017	3.04 ^b ±0.09	3.33 ^a ±0.031	3.42 ^a ±0.01	3.43 ^a ±0.02
5 (35)	3.56 ^a ±0.02	2.65 ^d ±0.023	3.22 ^c ±0.02	3.40 ^b ±0.01	3.48 ^{ab} ±0.03	3.57 ^a ±0.04
6 (42)	3.44 ^a ±0.07	2.46 ^d ±0.03	3.19 ^c ±0.01	3.26 ^b ±0.05	3.42 ^a ±0.07	3.45 ^a ±0.05

Other authors have also reported the decrease levels of serum albumen, total proteins and globulin in chick fed on ochratoxin contaminated diet only (Khadem *et al.*, 2012). No significant differences were observed among chicks of negative control and those fed on diets containing ochratoxin A plus adsorbent.

The results of broiler chicks in group D, E and F fed on 0.6%, 0.9% and 1.2% adsorbent respectively showed significant differences as compared to group B fed on ochratoxin A (400µg/kg feed) contaminated feed. However no significant difference was noted in the values of chicks in group A (kept on pure feed) and adsorbents treated groups. Similar findings were also presented previously (Kumar *et al.*, 2004). Our results demonstrated that the prepared adsorbents are highly efficient to adsorb and detoxify ochratoxin A in alimentary canal of *Gallus gallus domesticus* and passes along feces without any injurious effect.

Clinical signs and behavior

Scores of the different clinical signs and behavioral studies exhibited by different groups of chicks during each week of the experiment are given in Table (20s). Poultry birds in group A and D, E and F exhibited normal signs and behavior during the experiment. Abnormal signs and behavior were presented by birds in group B as this group exhibit less attraction toward feed and increased water intake throughout the experiment. Also the chicks in this group were depressed. The severity of clinical signs and behavior was at peak at the end of the experimental period. Normal signs and behavior were exhibited by chickens in group C during the first week. The results obtained in the 2nd week were similar to that of the 1st week with slight alterations in behavior, clinical signs and feces consistency. Intensity of clinical signs and behavior was increased with the increase in experimental duration.

Table 20s – Serum globulins concentration (g/dl) in broiler chickens kept on different levels of the prepared adsorbent derived from coconut shell.

Time Period	Group					
	Ochratoxin A 400µg/kg feed					
Week (days)	A	B	C	D	E	F
1 (07)	1.21 ^a ±0.017	1.06 ^b ±0.026	1.05 ^b ±0.028	1.12 ^{ab} ±0.0902	1.13 ^{ab} ±0.042	1.19 ^{ab} ±0.0441
2 (14)	1.21 ^{ab} ±0.041	1.09 ^b ±0.0467	1.14 ^{ab} ±0.047	1.18 ^{ab} ±0.026	1.18 ^{ab} ±0.0384	1.21 ^a ±0.0176
3 (21)	1.17 ^a ±0.0173	0.88 ^c ±0.0267	1.02 ^b ±0.06	1.15 ^{ab} ±0.0240	1.17 ^a ±0.030	1.19 ^a ±0.0319
4 (28)	1.22 ^a ±0.003	0.73 ^c ±0.024	1.0 ^b ±0.047	1.12 ^{ab} ±0.0203	1.21 ^{ab} ±0.017	1.22 ^a ±0.003
5 (35)	1.15 ^a ±0.024	0.55 ^c ± 0.026	1.07 ^b ±0.0208	1.12 ^{ab} ±0.0203	1.12 ^{ab} ±0.0291	1.16 ^a ±0.033
6 (42)	1.09 ^a ±0.068	0.43 ^b ±0.0406	1.02 ^a ±0.0291	1.03 ^a ±0.042	1.08 ^a ±0.062	1.09 ^a ±0.073



Different clinical signs and behavioral alterations in poultry chicks (during ochratoxicosis) have been reported by several researchers (Zahoor & Khan 2015). They also investigated that ochratoxin toxicity in broilers has resulted in increased water intake and

decreased feed consumption; therefore these results are also in accordance to our observed studies. According to our results diarrhea (watery stool), attraction to water intake and depression were some noticeable signs displayed by the broilers fed with

Table 21s – Clinical signs and behavior of broiler chickens kept on different concentration of the prepared adsorbent derived from coconut shell.

Time period	Clinical signs and behavior	Range of score	Groups					
			Ochratoxin A (400 µg/kg) of feed					
			A	B	C	D	E	F
First week	Alertness (normal/depressed)	0-3	0	2	0	0	0	0
	Attraction to feed (normal/less interest)	0-3	0	4	0	0	0	0
	Attraction to water (normal/more/less interest)	0-3	0	12	0	0	0	0
	Feces consistency (normal/ watery)	0-3	0	5	0	0	0	0
Cumulative score			0	23	0	0	0	0
Second week	Alertness (normal/depressed)	0-3	0	5	0	0	0	0
	Attraction to feed (normal/less interest)	0-3	0	9	0	0	0	0
	Attraction to water (normal/more/less interest)	0-3	0	22	1	0	0	0
	Feces consistency (normal/ watery)	0-3	0	13	3	0	0	0
Cumulative score			0	44	4	0	0	0
Third week	Alertness (normal/depressed)	0-3	0	7	1	0	0	0
	Attraction to feed (normal/less interest)	0-3	0	13	1	0	0	0
	Attraction to water (normal/more/less interest)	0-3	0	35	2	0	0	0
	Feces consistency (normal/ watery)	0-3	0	27	4	0	0	0
Cumulative score			0	82	8	0	0	0
Forth week	Alertness (normal/depressed)	0-3	0	12	1	0	0	0
	Attraction to feed (normal/less interest)	0-3	0	30	2	0	0	0
	Attraction to water (normal/more/less interest)	0-3	0	52	5	0	0	0
	Feces consistency (normal/ watery)	0-3	0	42	7	0	0	0
Cumulative score			0	136	15	0	0	0
Fifth week	Alertness (normal/depressed)	0-3	0	14	2	0	0	0
	Attraction to feed (normal/less interest)	0-3	0	48	4	0	0	0
	Attraction to water (normal/more/less interest)	0-3	0	71	7	0	0	0
	Feces consistency (normal/ watery)	0-3	0	62	10	0	0	0
Cumulative score			0	196	23	0	0	0
sixth week	Alertness (normal/depressed)	0-3	0	17	3	0	0	0
	Attraction to feed (normal/less interest)	0-3	0	70	6	0	0	0
	Attraction to water (normal/more/less interest)	0-3	0	86	9	0	0	0
	Feces consistency (normal/ watery)	0-3	0	84	10	0	0	0
Cumulative score			0	257	28	0	0	0
Total cumulative score			0	738	78	0	0	0



ochratoxin A contaminated feed. Such findings have also been observed by Koynarski *et al.* (2007) in chickens and laying hens fed with different dietary levels of ochratoxin. However adsorbent treated group have shown reduced cumulative score when compared to positive control group (B). Thus the present study clearly indicated the significant effect of prepared adsorbent (derived from coconut shell) in ameliorating the toxic effects of ochratoxin A in broiler chicks.

Relative percentage mortality

Table (21s) represented relative mortality of chicks, occurred among different groups at the end of each week of the experimental period (for six week). Maximum deaths occurred in B (positive control) that increased with the increase in experimental duration and was found severe at the end of the 6th week of the experiment. However the lowest rate of mortality was observed in the negative control group (fed with ochratoxin free basal feed). Adsorbent treated groups, particularly groups E and F also showed the lowest rate of mortality, like group A. The total percentage mortality was as follows: B> C> D> while A, E, F showed same percentage of mortality.

Increase in rate of mortality with increase in ochratoxin exposure duration and dietary OTA concentration (dose) in broilers has been reported by many studies (Elaroussi *et al.*, 2006). In the present study broilers in group B fed with ochratoxin A contaminated feed (without adsorbents) showed the highest percentage of total mortality as compared to adsorbent treated groups (C, D, E, F) and negative control group (A). These outcomes were in agreement with the conclusions of Zahoor *et al.* (2014). According to them, the highest percentage of mortality was observed in chicken exposed to different dietary levels of mycotoxin contaminated feed. However, least mortality was observed among groups fed with mycotoxin contaminated feed along with different levels of adsorbents.

Table 22s – Relative percentage mortality of broiler chickens kept on different concentrations of the adsorbent derived from coconut shell.

Time Period	Group					
	Ochratoxin A 400µg/kg feed					
Week (days)	A	B	C	D	E	F
1 (07)	0	0	0	0	0	0
2 (14)	1	2	0	1	0	0
3 (21)	0	3	1	0	0	1
4 (28)	1	7	3	1	2	0
5 (35)	1	13	5	3	1	1
6 (42)	0	8	3	1	0	1
Total mortality	3	33	12	6	3	3
% mortality	3%	33%	12%	6%	3%	3%

CONCLUSION

The comparative evaluation of the mentioned groups has indicated that when ochratoxin A contaminated feed, supplemented with different levels of adsorbent (prepared from coconut shell) was fed to broilers, the ochratoxin A induced signs and symptoms were prevented to great extent in chickens. The reason behind this is that most of the ochratoxin A is adsorbed from the intestinal track of broilers and passed along with feces without any injurious effects. Thus the prepared adsorbent has shown greater *in vivo* efficacy to ameliorate the toxic effects of OTA in poultry chicks.

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