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Haemato-biochemical studies on experimental ochratoxicosis in broiler: Its amelioration by fruit powder of *Tribulus terrestris* and toxin binder alone and in combination

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Abstract
The study was undertaken to evaluate the efficacy of *Tribulus terrestris* fruit powder (@ 1% of feed) and Toxin binder (Ingredient: Formulated Dipolar Phyllosilicates) (@ 1.5 gm/kg feed) given singly and in combination, in alleviating the toxic effects of ochratoxin A @ 800 ppb administered in broiler diet for 30 days. A total of 120 broiler birds were randomly divided into six equal groups, each comprising of 20 birds. Feeding of the OTA and its allied treatments commenced from 8th day of broiler age (after acclimatization period of 7 days; hence 8th day considered as 0 day). Haemato-biochemical parameters were studied on 15th and 30th days of the experiment.
In OTA fed birds (group I), haematological studies revealed significant reduction in the Haemoglobin, Packed Cell Volume, Total Erythrocyte Count, Total Leucocyte count and significant increase in the heterophil count (heterophilia) and blood clotting time, observed at both the intervals of study, while eosinophil and lymphocyte counts showed non-significantly increased and decreased mean values, respectively, whereas basophil and monocyte counts remained unaltered throughout the study. Biochemical profiles revealed significant decrease in serum total protein, albumin and globulin, whereas significant increase in the serum levels of ALT, AST, ALP, BUN, uric acid and creatinine at both the intervals of study. However inclusion of *T. terrestris* (group IV) and toxin binder (group V) found to be effective in reducing toxic effects of OTA either singly or in combination of both. Further, combination of both the treatment (group VI) showed better improvement in all the altered haemato-biochemical values than their single treatment.

Keywords: *Tribulus terrestris* fruit powder, toxin binder, ochratoxin A @ 800 ppb, haemato-biochemical

Introduction
Poultry industry has been one of the fastest expanding industries in the world. Presently, India is largest broiler producer and ranks second in Asia and fourth in the world (INCRA Ltd.). A hurdle to this constant hike in poultry production has been outbreaks of emerging and re-emerging diseases. Mycotoxicosis is one of the most dreadful and constant problem amongst the microbial diseases. Mycotoxins are biologically active, secondary metabolites produced by toxigenic fungi mainly belonging to *Aspergillus* spp., *Fusarium* spp., *Penicillium* spp. and *Claviceps* spp [1]. Amongst these, aflatoxin and ochratoxin are the major and most common mycotoxins found in poultry. In the tropical countries like India, where prevalence of moderate to high ambient temperature with high relative humidity and moisture levels, incidences and ill effects of such mycotoxins are widely experienced. Toxicological spectrum of various mycotoxins is very wide encompassing different kind of toxicities *viz*. acute and chronic toxicities, nephrotoxicity, hepatotoxicity, immunosuppression, embryotoxicity, carcinogenicity, genotoxicity, mutagenicity and teratogenicity in animals and poultry [2]. In view of considerable economic implications involved and the hazardous effects of mycotoxin residues in animal products on human health, considerable attention is needed to develop alternative pathways and methodologies to counteract the problem of ochratoxicosis. Practically suitable and cost effective methods to counteract mycotoxicosis in poultry feed are in great demand. Therefore, the present work was undertaken to study the ameliorative effects of *Tribulus terrestris* fruit powder and Toxin binder against induced ochratoxicosis.

Materials and Methods

Experimental chicks: For this experiment, a total of 120 healthy day old Marek's disease vaccinated 'Vencobb 400' commercial broiler chicks were purchased from M/s Venkateshwara Hatcheries Private Limited, Pune.

Fungal culture: Pure culture of *Aspergillus ochraceus* was obtained from Microbial Type Culture Collection and Gene Bank (MTCC), Chandigarh. After sub-culturing on Czapek agar medium (Hi-media), this culture was processed on mixture of grains (substrate) to yield required quantity of Ochratoxin needed for the experimental trial.

***Tribulus terrestris* fruit powder:** The well quality air dried, *Tribulus terrestris* fruits (Gokhru) were procured from local market. Then these fruits were broken manually, powdered using electric grinder and then this fruit powder was added in poultry feed @ 1% of feed against induced ochratoxicosis.

Toxin binder: A broad spectrum, locally available mycotoxin binder, containing combination of preselected dipolar phyllosilicates procured from local market, was used as a mycotoxin binder for this study. It was added in the feed at the level of 1.5 gm/kg feed, as recommended by the manufacturer.

Ochratoxin A production and analysis: Ochratoxin A was produced on substrate containing unpolished rice (20%), maize (30%), soyabean (30%) and groundnut (20%) by using already procured culture of *Aspergillus ochraceus.* Change in color from white to sulphur color and then blackish was noted. The fungus culture thus grown on substrate was autoclaved, dried at 50º- 70 ºC in hot air oven for overnight and then grounded to a fine powder form. Around 50-100 gms of this powered mouldy substrate was analyzed for quantification of OTA by thin layer chromatoghraphy (TLC), at Mycotoxin Laboratory, Central Avian Research Institute, Izzatnagar (UP).

Experimental feed: Scientifically prepared, well quality commercial poultry feed, free of toxin binder and moulds, containing adequate nutrients in proper balanced ratio with its specification for each age group such as pre-starter, starter and finisher was purchased from M/s VRK Nutrional Solutions, Sangli (MS). This feed was offered to broiler chicks from 0-7th days, 8th-15th days and 16th-38th day of age as per age group such as pre-starter, starter and finisher, respectively. Powdered Ochratoxin, fruit powder of *T. terrestris* and toxin binder were added in the normal feed to obtain a level of 800 ppb, 1% and 1 gm per kg of feed, respectively.

Experimental procedure: A total of 120 broiler birds were randomly divided into six equal groups (Table 1), each comprising of 20 birds. The group I was fed with normal feed, group II with Ochratoxin A @ 800 ppb and group III with 1% of *T. terrestris* powder, group IV with OTA @ 800 ppb and *T. terrestris* @ 1% of feed, group V with OTA @ 800 ppb and Toxin binder @ 1.5 gm/kg of feed and group VI with combination of 1% fruit powder of *T. terrestris* and 1.5 gm/kg of feed toxin binder daily through feed for a period of 30 days.

Table 1 Details of experimental groups

Sr. No.	Group	Treatment	Birds / Group
1.	Group I	Healthy control	20
2.	Group II	Ochratoxin @ 800 ppb through feed	20
3.	Group III	Dried powder of *Tribulus terrestris* plant @ 1% of feed through feed	20
4.	Group IV	Ochratoxin @ 800 ppb + Dried powder of *Tribulus terrestris* plant @ 1% of feed through feed	20
5.	Group V	Ochratoxin @ 800 ppb + Toxin binder* @1.5 gm/kg of feed through feed	20
6.	Group VI	Ochratoxin @ 800 ppb + Dried powder of *Tribulus terrestris* plant @ 1% of feed + Toxin binder* @ 1.5 gm/kg of feed through feed	20
	Total		120

* Locally available market drug, containing combination of preselected dipolar phyllosilicates

Haemato-biochemical estimations: Blood was collected on 0 day, 15th and 30th day of experiment (in anticoagulant vials for haematology and in clot activator vials for serum biochemistry after collection of separated serum) and processed for estimation of different haemato-biochemical parameters (pre and post-treatment intervals).

Ethical approval: The study was approved by the IAEC.

Statistical analysis: The data generated from haemato-biochemical estimations at different intervals of experiment in each group were statistically analyzed by using Completely Randomized Design (CRD) to determine the means and standard error of each parameter and a probability of $P<0.05$ was accepted as significant [3].

Results and Discussion

Haematological changes: Average haematological values in broiler chicks of all the experimentalgroupsobserved at 0, 15th and 30th days of experiment are presented in Table 2 and 3.

Haematological estimations such as Haemoglobin (Hb), Packed Cell Volume (PCV), Total Erythrocyte Count (TEC) and Total Leucocyte Count (TLC) were found to be significantly decreased in the ochratoxin affected birds as compared to healthy birds, whereas, there was significant raised values of heterophils (heterophilia) and blood clotting time in OTA fed birds, when compared to respective values in healthy birds. Alterations in Hb, PCV, TEC, TLC, heterophil count and blood clotting time were found to be partially improved in all treatment groups (group IV, V and VI) with better improvement in birds of combined treatment group (group VI). Similar observations have been supported by various researchers [4-6].

Reduction in the Hb concentration in the ochratoxicated birds in present study might be due to depressed erythropoiesis resulting into anaemic condition. Anaemia in the ochratoxin fed birds previously reported by many researchers [7-8]. Anaemia developed in ochratoxin fed birds might be as a result of depressed erythropoiesis due to nephropathy and defective protein metabolism due to hepatopathy [9]. In

addition, this reduction in Hb concentration may be attributed to the reduced protein synthesis, inhibition of protein synthesis [10] and impaired iron absorption combined with suppression of haemopoiesis [11].

Decreased PCV levels could be due to iron deficiency or direct harmful effects of OTA on haemopoiesis [4], anaemia developed as a result of depressed erythropoiesis due to nephropathy and defective protein metabolism due to hepatopathy [9] and impaired iron absorption combined with suppression of haemopoiesis [11].

Decreased RBC count observed in the present study could be due to depressed erythropoiesis due to nephropathy and defective protein metabolism due to hepatopathy [9] and oxidative effects of OTA on RBCs by lowering its phosphoenolpyruvate kinase which may produce energy deficient erythrocytes [12]. In addition, ochratoxin produces its toxic effects through apoptosis, disruption of cytoskeleton or mitochondrial respiration [13].

Reduction in the WBC count could be due to direct effects of OTA on germinal centres of lymphoid tissues and function alterations in the immune response [4, 14]. In addition, ochratoxin has lymphotoxic effects on leucocyte which could also be a reason of reduced leucogram in ochratoxicosis.

Table 2: Haematological values (Mean ± S.E.) in experimental chicks of different groups at various intervals

Sr. No	Parameters	Interval (d)	Group I	Group II	Group III	Group IV	Group V	Group VI
1.	Haemoglobin (gm/dl)	0 day	9.90 ± 0.16	9.83 ± 0.17	9.86 ± 0.22	9.80 ± 0.23	9.80 ± 0.23	9.83 ± 0.27
		15th day	9.93 ^{a} ± 0.28	7.70 ^{b} ± 0.16	9.86 ^{a} ± 0.32	8.20 ^{b} ± 0.29	8.26 ^{b} ± 0.27	8.33 ^{b} ± 0.18
		30th day	10.36 ^{a} ± 0.29	07.93 ^{c} ± 0.14	09.83 ^{a} ± 0.38	08.53 ^{bc} ± 0.17	08.56 ^{bc} ± 0.20	08.66 ^{b} ± 0.22
2.	Packed Cell Volume (%)	0 day	31.16 ± 1.10	30.83 ± 0.90	30.83 ± 1.22	30.66 ± 0.95	30.50 ± 1.40	30.33 ± 0.95
		15th day	31.00 ^{a} ± 1.15	26.16 ^{b} ± 0.94	30.33 ^{a} ± 1.05	28.00 ^{ab} ± 1.06	28.16 ^{ab} ± 1.35	28.33 ^{ab} ± 0.76
		30th day	31.33 ^{a} ± 1.05	25.66 ^{b} ± 1.25	31.00 ^{a} ± 1.41	27.50 ^{b} ± 0.84	28.33 ^{ab} ± 0.91	28.50 ^{ab} ± 0.84
3.	Total Erythrocyte Count(millions/mm^3)	0 day	3.02 ± 0.14	3.02 ± 0.07	3.05 ± 0.06	3.09 ± 0.04	3.08 ± 0.06	3.10 ± 0.09
		15th day	3.10 ^{a} ± 0.08	2.10 ^{b} ± 0.09	2.99 ^{a} ± 0.15	2.24 ^{b} ± 0.11	2.30 ^{b} ± 0.10	2.35 ^{b} ± 0.09
		30th day	3.19 ^{a} ± 0.09	2.04 ^{b} ± 0.07	3.06 ^{a} ± 0.09	2.37 ^{b} ± 0.13	2.24 ^{b} ± 0.12	2.29 ^{b} ± 0.18
4.	Total Leucocyte Count (thousand/mm^3)	0 day	22.11 ± 0.84	22.81 ± 0.82	22.56 ± 0.80	22.23 ± 0.65	22.71 ± 0.41	22.77 ± 0.76
		15th day	23.19 ^{a} ± 0.80	18.77 ^{c} ± 0.69	23.11^{a} ± 0.39	20.55 ^{bc} ± 0.78	20.86 ^{b} ± 0.59	21.02 ^{b} ± 0.86
		30th day	23.28 ^{a} ± 0.63	19.27 ^{b} ± 0.75	23.23 ^{a} ± 0.98	20.78 ^{b} ± 0.47	20.92 ^{b} ± 0.56	21.14 ^{b} ± 0.50
5.	Blood Clotting Time (Sec)	0 day	65.59 ± 1.33	65.17 ± 0.96	66.39 ± 0.92	65.40 ± 0.94	66.02 ± 1.01	65.93 ± 1.21
		15th day	67.22 ^{c} ± 1.26	94.56 ^{a} ± 2.89	67.11 ^{c} ± 1.09	84.71 ^{b} ± 1.00	84.28 ^{b} ± 1.26	81.80 ^{b} ± 1.14
		30th day	66.46 ^{c} ± 1.75	104.09 ^{a} ± 2.96	64.03 ^{c} ± 2.01	97.66 ^{b} ± 0.86	98.02 ^{ab} ± 2.65	94.08 ^{b} ± 1.82

*Means bearing similar superscripts within the columns do not differ significantly (P< 0.05)

Elevation in the BCT in ochratoxin affected birds has been recorded by various researchers [5, 15, 16-17]. However, one of the researchers observed marginally raised blood clotting time in the birds fed with 100 ppb and 200 ppb OTA [12]. Significant increase in the blood clotting time in the OTA fed birds was possibly due to the impaired blood coagulation (both extrinsic and intrinsic mechanisms) and severe coagulopathy primarily due to hypofibrinogenemia [12-18].

Table 3: Differential Leucocyte Counts (Mean ± S.E.) in experimental chicks of different groups at various intervals

Sr. No.	Parameters	Intervals (d)	Group I	Group II	Group III	Group IV	Group V	Group VI
1.	Heterophil (%)	0 day	25.00 ± 0.85	25.66 ± 0.88	25.33 ± 1.02	25.50 ± 0.80	25.50 ± 0.76	24.66 ± 0.49
		15th day	24.83 ^{b} ± 0.54	29.33 ^{a} ± 0.84	25.50 ^{b} ± 1.02	26.66 ^{b} ± 0.84	26.16 ^{b} ± 0.79	26.00 ^{b} ± 0.85
		30th day	25.16 ^{b} ± 1.49	28.50 ^{a} ± 0.67	24.16 ^{b} ± 0.47	26.33 ^{ab} ± 0.71	26.16 ^{ab} ± 0.70	26.16 ^{ab} ± 0.98
2.	Eosinophil (%)	0 day	2.16 ± 0.30	2.66 ± 0.42	2.83 ± 0.30	2.66 ± 0.33	2.66 ± 0.33	2.83 ± 0.47
		15th day	2.66 ± 0.33	4.16 ± 0.47	2.66 ± 0.33	3.66 ± 0.33	3.33 ± 0.42	3.33 ± 0.49
		30th day	2.50 ± 0.34	4.33 ± 0.71	2.83 ± 0.40	3.83 ± 0.30	3.50 ± 0.42	3.33 ± 0.42
3.	Basophil (%)	0 day	0.33 ± 0.21	0.33 ± 0.21	0.50 ± 0.34	0.33 ± 0.21	0.33 ± 0.33	0.50 ± 0.22
		15th day	0.50 ± 0.22	0.16 ± 0.16	0.33 ± 0.21	0.33 ± 0.21	0.33 ± 0.21	0.50 ± 0.22
		30th day	0.50 ± 0.22	0.33 ± 0.21	0.50 ± 0.22	0.50 ± 0.22	0.50 ± 0.22	0.16 ± 0.16
4.	Lymphocyte (%)	0 day	66.66 ± 1.74	65.33 ± 0.98	65.00 ± 1.31	65.16 ± 1.57	65.66 ± 0.61	65.83 ± 0.54
		15th day	66.00 ± 1.12	62.00 ± 0.73	66.66 ± 1.11	63.66 ± 1.33	64.83 ± 0.87	64.33 ± 1.40
		30th day	65.33 ± 1.90	62.33 ± 0.88	65.66 ± 0.71	63.50 ± 0.67	64.33 ± 1.14	64.33 ± 0.88
5.	Monocyte (%)	0 day	5.83 ± 0.83	6.00 ± 0.44	6.16 ± 0.47	5.66 ± 0.49	5.83 ± 0.30	5.83 ± 0.60
		15th day	5.83 ± 0.40	5.33 ± 0.49	5.50 ± 0.50	5.33 ± 0.49	5.33 ± 0.66	5.66 ± 0.61
		30th day	6.33 ± 0.49	5.50 ± 0.42	6.16 ± 0.40	5.83 ± 0.60	5.66 ± 0.49	5.83 ± 0.47

*Means bearing similar superscripts within the columns do not differ significantly (P< 0.05)

Increase in the Heterophil count in ochratoxin affected birds in the present study are in agreement with the observations of previous researchers [14, 16-19] who recorded significant heterophilia in the broilers fed with ochratoxin A. However, some reported heterophilia at early period (14th day), whereas inconsistent heterophilia at later stages (28th and 42nd) of ochratoxicosis by Ochratoxin A @ 1 ppm in broilers [5]. Significantly increased Heterophil count at 15th day could be due to normal inflammatory response shown by the birds whereas later on numerical decrease at 30th day suggest the suppressing effect of OTA on lymphoid tissues which alter the proliferation and differentiation of lymphocytes [12-13].

Mean ± S. E. values of Basophil and Monocyte count did not alter, However there were non-significant increase and decrease in the mean values of Eosinophil and Lymphocyte, respectively at all intervals of study. Contrary to this, one researcher found no alteration in the Eosinophil, Lymphocyte and Monocyte count in the ochratoxin fed birds at all intervals

of study in the 1 ppm fed ochratoxicated birds [5]. However, some researchers found mild lymphocytopenia and mild eosinophilia in the OTA fed birds at the level of 1 ppm at all intervals of study [16], whereas others reported mild monocytopenia in the ochratoxin fed birds at the inclusion level of 2 ppm and 3 ppm OTA, respectively [14-19].

Biochemical changes: Average biochemical values in broiler chicks of all the experimental groups observed at 0, 15th and 30th days of experiment are presented in Table 4.

There was significant decline in the mean serum biochemical values of total protein, albumin and globulin in the birds of IInd group (800 ppb OTA group) as compared to values in control group indicating liver damage and renal leakage of protein, whereas, there was significant elevation in mean serum levels of ALT, AST, ALP, BUN, uric acid and creatinine in birds of OTA fed group as respect to healthy group observed at 15th and 30th day indicating kidney damage in OTA group birds. There was partial improvement in the altered mean values of total protein, albumin, globulin, ALT, AST, ALP, BUN, uric acid and creatinine in the birds of group IV, V and VI, however better improvement was observed in group VI.

Several workers reported significantly increased serum levels of ALT and AST in ochratoxicosis by OTA [20, 21, 22-23]. Findings of present study are in consonance with these reports of earlier workers. Increased serum ALP values in the broilers fed with ochratoxin A were also recorded previously [6,21&23]. Increased serum levels of ALT, AST and ALP was attributed to cellular damage, degenerative changes produced in the liver and increased plasma membrane permeability resulting leakage of enzymes into serum [21-24].

Various researchers reported significantly decreased serum levels of total protein, albumin and globulin in ochratoxicosis by OTA [6, 9, 16, 20, 21-22]. The mechanism by which OTA produced hypoproteinaemia, hypoalbuminaemia and hypoglobulinaemia is due to inhibition of phenylalanyl transfer-RNAsynthetase enzyme with phenylalanine resulting into inhibition of hepatic protein synthesis and renal leakage of both proteins resulting from kidney lesions induced by OTA [25].

Previous workers reported significantly higher BUN values in ochratoxicosis [6-21]. Findings of present study go parallel with these reports of earlier workers. In contrast to this, some observed significantly decreased BUN values in the OTA fed birds [22]. Increased levels of BUN can be attributed to the inflammatory and degenerative changes in the kidney (nephrotoxicity) caused by OTA [21].

There were previously reported significantly increased serum levels of uric acid and creatinine in ochratoxin fed birds [6, 19, 23-26]. These increased levels of uric acid and creatinine in ochratoxin affected birds might be due to impaired renal function through destruction of nephrocytes of PCT, collecting tubules and even glomeruli [19-21].

Table 4: Biochemical values (Mean ± S.E.) in experimental chicks of different groups at various intervals

Sr. No.	Parameters	Interval (d)	Group I	Group II	Group III	Group IV	Group V	Group VI
1.	ALT (IU/L)	0 day	11.00 ± 0.25	11.05 ± 0.21	11.03 ± 0.29	10.95 ± 0.21	10.96 ± 0.24	10.98 ± 0.28
		15th day	11.21 ^{d} ± 0.21	15.54 ^{a} ± 0.29	11.25 ^{d} ± 0.25	13.94 ^{b} ± 0.20	13.86 ^{bc} ± 0.17	13.20 ^{c} ± 0.23
		30th day	11.34 ^{d} ± 0.28	17.13 ^{a} ± 0.19	11.39 ^{d} ± 0.23	15.44 ^{b} ± 0.28	14.93 ^{bc} ± 0.20	14.23 ^{c} ± 0.30
2.	AST (IU/L)	0 day	167.74 ± 0.29	167.80 ± 0.32	167.70 ± 0.30	167.79 ± 0.40	167.71 ± 0.46	167.76 ± 0.46
		15th day	168.23 ^{d} ± 0.83	188.29 ^{a} ± 0.90	169.12 ^{d} ± 0.68	179.96 ^{b} ± 0.60	178.99 ^{b} ± 1.00	175.98 ^{c} ± 0.65
		30th day	169.78 ^{d} ± 1.07	192.21 ^{a} ± 1.09	170.44 ^{d} ± 0.94	181.00 ^{b} ± 1.22	179.55 ^{bc} ± 1.02	177.76 ^{c} ± 1.14
3.	ALP (IU/L)	0 day	152.13 ± 0.83	151.57 ± 0.85	150.48 ± 0.55	152.47 ± 0.59	150.17 ± 1.20	151.43 ± 0.85
		15th day	153.25 ^{e} ± 0.96	173.34 ^{a} ± 0.89	153.72 ^{e} ± 0.64	166.89 ^{b} ± 0.82	163.77 ^{c} ± 1.08	160.33 ^{d} ± 0.65
		30th day	155.31 ^{d} ± 1.06	178.83 ^{a} ± 0.95	154.65 ^{d} ± 0.85	167.26 ^{b} ± 0.99	166.70 ^{bc} ± 0.88	164.10 ^{c} ± 0.56
4.	Total Protein (gm/dl)	0 day	4.04 ± 0.21	4.00 ± 0.21	3.96 ± 0.28	4.04 ± 0.21	4.07 ± 0.33	4.01 ± 0.22
		15th day	4.10 ^{a} ± 0.08	2.91 ^{b} ± 0.09	4.09 ^{a} ± 0.18	3.19 ^{b} ± 0.18	3.28 ^{b} ± 0.22	3.25 ^{b} ± 0.12
		30th day	4.19 ^{a} ± 0.12	3.08 ^{b} ± 0.15	4.23 ^{a} ± 0.10	3.31 ^{b} ± 0.14	3.36 ^{b} ± 0.19	3.36 ^{b} ± 0.11
5.	Albumin (gm/dl)	0 day	1.75 ± 0.07	1.77 ± 0.08	1.70 ± 0.10	1.74 ± 0.05	1.76 ± 0.04	1.75 ± 0.03
		15th day	1.80 ^{a} ± 0.07	1.29 ^{c} ± 0.07	1.80 ^{a} ± 0.12	1.45 ^{bc} ± 0.05	1.47 ^{b} ± 0.04	1.48 ^{b} ± 0.10
		30th day	1.82 ^{a} ± 0.06	1.38 ^{b} ± 0.04	1.84 ^{a} ± 0.10	1.55 ^{b} ± 0.11	1.56 ^{b} ± 0.07	1.56 ^{b} ± 0.09
6.	Globulin (gm/dl)	0 day	2.28 ± 0.13	2.23 ± 0.10	2.26 ± 0.16	2.29 ± 0.13	2.31 ± 0.24	2.26 ± 0.15
		15th day	2.30 ^{a} ± 0.08	1.62 ^{c} ± 0.13	2.29 ^{ab} ± 0.21	1.74 ^{c} ± 0.15	1.81 ^{bc} ± 0.22	1.77 ^{c} ± 0.15
		30th day	2.37 ^{a} ± 0.19	1.69 ^{b} ± 0.16	2.39 ^{a} ± 0.12	1.76 ^{b} ± 0.18	1.80 ^{b} ± 0.16	1.77 ^{b} ± 0.14
7.	BUN (mg/dl)	0 day	5.09 ± 0.16	5.08 ± 0.13	4.98 ± 0.11	5.13 ± 0.08	5.10 ± 0.13	5.04 ± 0.09
		15th day	5.18 ^{c} ± 0.07	6.70 ^{a} ± 0.17	5.20 ^{c} ± 0.06	6.14 ^{b} ± 0.10	6.15 ^{b} ± 0.13	6.01 ^{b} ± 0.12
		30th day	5.25 ^{c} ± 0.07	6.83 ^{a} ± 0.12	5.23 ^{c} ± 0.14	6.22 ^{b} ± 0.20	6.10 ^{b} ± 0.18	6.04 ^{b} ± 0.18
8.	Uric Acid (mg/dl)	0 day	6.25 ± 0.11	6.28 ± 0.15	6.21 ± 0.12	6.23 ± 0.12	6.29 ± 0.15	6.22 ± 0.10
		15th day	6.30 ^{c} ± 0.22	11.26 ^{a} ± 0.28	6.32 ^{c} ± 0.15	9.32 ^{b} ± 0.32	9.19 ^{b} ± 0.25	8.98 ^{b} ± 0.26
		30th day	6.31 ^{d} ± 0.14	12.27 ^{a} ± 0.29	6.30 ^{d} ± 0.13	9.91 ^{b} ± 0.17	9.62 ^{bc} ± 0.39	9.19 ^{c} ± 0.25
9.	Creatinine (mg/dl)	0 day	0.43 ± 0.02	0.42 ± 0.02	0.42 ± 0.01	0.43 ± 0.02	0.44 ± 0.02	0.44 ± 0.02
		15th day	0.45 ^{bc} ± 0.02	0.59 ^{a} ± 0.02	0.44 ^{c} ± 0.01	0.51 ^{ab} ± 0.03	0.52 ^{ab} ± 0.01	0.51 ^{bc} ± 0.02
		30th day	0.46 ^{bc} ± 0.02	0.60 ^{a} ± 0.02	0.45 ^{c} ± 0.01	0.53 ^{ab} ± 0.03	0.53 ^{ab} ± 0.02	0.51 ^{bc} ± 0.02

*Means bearing similar superscripts within the columns do not differ significantly (P<0.05)

In conclusion, Ochratoxin @ 800 ppb daily fed through feed caused significant reduction in Hb, PCV, TEC and TLC levels while significant increase in heterophil count (heterophilia) and significantly delayed blood clotting time in OTA fed birds reflected its toxic effects on haemopoietic system. Also, severely altered serum biochemical observations of total protein, albumin, globulin, ALT, AST, ALP, BUN, uric acid and creatinine in birds of OTA fed group support the fact of nephrotoxic and hepatotoxic effects of ochratoxin. However, addition of 1% fruit powder of *Tribulus terrestris* daily for 30 days in the feed of ochratoxicated birds had partial ameliorative effects against 800 ppb OTA level. When comparison between three treatments was made, it was observed that the combined treatment (plant powder + toxin binder) had better ameliorative effects than alone treatment of plant powder or toxin binder.

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