

## Effect of Herbal Mycotoxin Binders in Amelioration of Induced Mycotoxicosis in White Leghorn Laying Hens

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### Abstract

Efficacy of herbal mycotoxin binders in ameliorating induced mycotoxicosis was evaluated in white leghorn laying hens. Birds were randomly divided into six groups containing 15 birds in each group. Group I was served as control fed with basal diet, group II birds were fed with aflatoxins and ochratoxin A at 100 ppb each. Group III, IV, V and VI birds were fed with aflatoxins and ochratoxin A at 100ppb each and herbal mycotoxin binders Vilocym<sup>®</sup>, Toxiroak<sup>®</sup>, Vilocym-Z<sup>®</sup> in feed at 1 kg/tonne and AV/LBP/20<sup>®</sup> at 1 ml/litre in drinking water respectively for 10 weeks. The cultured rice and wheat samples were screened for presence of mycotoxins by LC-MS/MS method. Aflatoxins concentration in cultured rice sample was 826 ppb. Ochratoxin A concentration in cultured wheat sample was 8990 ppb. The hematological parameters viz., Hb, TEC, PCV showed significant decreased level in (Group II, III, IV, V and VI) compared to their respective control group. Similarly biochemical parameters viz., aspartate aminotransferase, alanine aminotransferase showed significantly decreased level in treated groups (Group II, III, IV, V and VI) compared to their respective control group. Serum albumin and serum total protein level significantly decreased in treated groups (Group II, III, IV, V and VI) compared to their respective control group (Group I). Histopathology of Group II birds revealed toxic effects on liver and kidney. Supplementation of herbal mycotoxin binders in mycotoxicated feed showed improvement in all the parameters indicating that herbal mycotoxin binders reduce the severity of toxicity.

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### INTRODUCTION

Poultry industry which provides cheap source of animal protein has taken a quantum leap in the last three decades evolving from a near backyard practice to a venture of industrial promotion. Poultry is one of the fastest growing segments of the agricultural sector in India with rising rate of 8 per cent per annum and it is the major contributor of growing economy of the country. India stands fifth in the world egg producing countries with 55.6 billion eggs production during 2008. Therefore, the problems of feed related disorders have been given considerable attention in recent years. The quality of feed and feed ingredients plays an important role in determining the performance of birds and profit margin to the poultry farmers. In tropical countries like India, prevalence of high moisture and humid condition, poor harvesting techniques, inadequate drying facilities and unseasonal rains have provided conducive environment for fungal invasion of feed resulting in proliferation and elaboration of toxic metabolites called as mycotoxins. Among mycotoxins, aflatoxin and ochratoxin are the most common and simultaneous contaminants of feed stuffs. Aflatoxins (AF) are potent hepatotoxic and immunosuppressive agents produced by *Aspergillus parasiticus* and *Aspergillus flavus*. Aflatoxicosis is an

acute or chronic condition characterized by growth retardation, reduced feed consumption, decreased body weight and immune suppression, decreased egg quality and production (Ghosh *et al.*, 1990). Ochratoxins (OA) are a group of structurally related toxic fungal metabolites, which are potent nephrotoxic agents chiefly produced by *Aspergillus ochraceus*. Ochratoxicosis resulting from the ingestion of ochratoxin is an acute or chronic condition characterized by decrease in production performance, egg quality, growth retardation, decreased feed consumption, immune suppression and mortality (Sawale *et al.*, 2009). AF and OA could interact synergistically and adversely affect the performance and productivity of birds.

Efforts have been made in the past to counteract the toxic effects of these toxins in the feed by use of physical, chemical and biological methods with limited success. Deleterious effects of aflatoxin could be overcome, or at least diminished, by adsorbents (Raju and Devegowda, 2000). Stoev *et al.* 2000 and Kurkure *et al.* (2000) reported ameliorating effect of Artichoke and *Curcuma longa* (turmeric) powder on ochratoxin A and aflatoxin B1. However, published literature on protective role of herbal toxin binders in mycotoxicosis induced by combination of

AF and OA is scanty. Henceforth, present study was designed to study the protective role of four herbal mycotoxin binder products in reduction of mycotoxicosis induced by combined effect of AF and OA in white leghorn laying hens for ten week.

## MATERIALS AND METHODS

*Aspergillus flavus* (MTCC 2798) and *Aspergillus ochraceus* (MTCC 10276) cultures were obtained from Microbial Type Culture Collection and Gene Bank (MTCC), Institute of Microbial Technology (IMTECH), Chandigarh, India. Potato Dextrose Agar (PDA) was procured from Himedia (Mumbai, India). Acetonitrile, formic acid, n-hexane, ammonium acetate buffer and methanol were used of HPLC grade Merck Chemicals. The mycotoxin standards like aflatoxins (B1, B2, G1 and G2), ochratoxin A, HT-2 toxin, diacetoxyscirpenol (DAS), deoxynivalenone (DON), 3-acetyldeoxynivalenol (3-AcDON), 15-acetyldeoxynivalenol (15-AcDON), beauvericin, apicidin and citrinin were obtained from Sigma Aldrich Chemicals (Mumbai, India). T-2 toxin, fumonisin B1, fumonisin B2 and zearalenone were also used (Ermentek, Israel).

Aflatoxin was produced on polished rice using *A. flavus* (MTCC 2798) culture suggested by Shotwell *et al.* (1966). Ochratoxin was produced on wheat using *A. ochraceus* (MTCC 10276) culture suggested by Trenk *et al.* (1971). The rice was soaked in water for two hours and water for overnight and autoclaved at 15 psi for 15 min and then each was inoculated with 0.5ml of respective fungal spore suspension. The inoculum was incubated for seven days at room temperature in dark place with vigorous shaking from third day after inoculation. On seventh day, the flasks with green coloured mycelial growth were autoclaved at 121°C (15 psi) for 20 min. The fermented rice and wheat was collected and dried overnight in a hot air oven at 80°C. The dried material was powdered and stored in a dark place.

### Extraction of Toxin

Twenty one days old fungal cultured rice and wheat material was ground to fine powder in mixer and dried overnight in hot air oven at 60°C. After that, 5 g of sample was extracted for six hour with 25.00mL mixture of Acetonitrile/formic acid in water/hexane (80:19:01) at 25°C under constant agitation. Extract was spun for 1:00 hour and the lower aqueous Acetonitrile layer (4.00mL) was dried in a water bath under nitrogen streaming. The sample was dispersed in 4.00 mL 10mM ammonium acetate buffer (pH 8.5)/methanol (40:60) mixture. The sample (2.00mL) was cleaned up using OASIS® - HLB cleanup SPE cartridge. The purified sample was dried and reconstituted in 0.500mL of HPLC mobile phase (10mM ammonium acetate (pH 8.5)/methanol (40:60%v/v) and filtered through hydrophilic PTFE membrane syringe filters (20µm) to auto sampler vial.

### Analysis of Mycotoxins

The extracts were analyzed for the presence of aflatoxins (B1, B2, G1 and G2), ochratoxin, trichothecenes, fumonisins, beauvericin, apicidin, zearalenone and citrinin by LC-MS/MS method. HPLC 1200 series (Agilent Technologies, Waldbron, Germany) coupled to Applied Biosystems (USA), MDS Sciex 3200 Q Trap LC-MS/MS System was used. HPLC column of Zorbax, Eclipse XDD-C 18, resolution, HT 4.6x50mm, 1.8micron, 600 Bar column was used (Agilent Technologies, Waldbron, Germany). The presence of 17 different mycotoxins was monitored using LC-MS/MS. Mycotoxin identity confirmation was performed by monitoring of at least two transitionions per analyte. The HPLC system utilized a binary gradient solvent system consisting of nanopure grade RO water buffered by 10mM ammonium acetate (pH 8.5) and methanol (40:60%v/v). The total run time was 28 minutes per sample analyzed.

Quantification of toxins was carried out from standard calibration curve of each toxin. Calibration curves were built individually using 5 or 6 concentration levels in maize matrix (Mycotoxin free) for each mycotoxin analyzed. The peak area of the mycotoxins was plotted against the concentration to generate matrix matched calibration curve.

White leghorn laying hens (BV-300) of 25 weeks age were procured from a private poultry farm. Birds were acclimatized for one week and then randomly divided into six groups containing 15 birds in each group. Vaccination was done as per routine farm practice. Layer mash feed was procured from Department of Poultry Science, Veterinary College, KVAFSU, Hebbal, Bangalore, India. The feed sample was tested for mycotoxin levels before feeding to birds.

Powdered AF and OA culture materials were incorporated into the feed so as to give final concentration of 100 ppb each. Four different toxin binder formulations were obtained from Ayurved limited, Himachal Pradesh, India and were mixed in the feed at the rate of one kg/per tonne of feed. Four different natural toxin binders were as follows;

1. Toxiroak® – *Allium sativum*, *Azadirachta indica*, *Solanumnigrum*, *Emblicoefficialis*, *Curcuma longa* and hydrated soduim calcium aluminosilicates (HSCAS).
2. Vilocym-Z® - *Solanumnigrum*, *Citrulluscolocynthis*, *Phyllanthusemblica* and *Curcuma longa*.
3. Vilocym®- *Solanumxanthocarpum*, *Emblicoefficialis*, *Ashwagandha* and *Azadirachta indica*.
4. AV/LBP/20® - *Azadirachta indica*, *Andrographis- paniculata*, *Citrulluscolocynthis* and *Emblicoefficialis*.

Birds were feed as per the protocol shown in Table 1. Control consist of birds feed without toxins and toxin binder.

**Table 1:** Birds feed treatment with and without toxins or toxin binder

| Groups | Feed Treatments       | Aflatoxin (ppb) | Ochratoxin (ppb) | Toxin Binder (kg/ tone feed) | Number of Birds |
|--------|-----------------------|-----------------|------------------|------------------------------|-----------------|
| I      | Control               | -               | -                | -                            | 15              |
| II     | Toxins without binder | 100             | 100              | -                            | 15              |
| III    | Toxins + Vilocym®     | 100             | 100              | 1                            | 15              |
| IV     | Toxins + Toxiroak®    | 100             | 100              | 1                            | 15              |
| V      | Toxins + Vilocyn-Z®   | 100             | 100              | 1                            | 15              |
| VI     | Toxins + AV/LBP/20®   | 100             | 100              | 1                            | 15              |

Haematology parameters like total erythrocyte count (TEC), haemoglobin, packed cell volume (PCV) were estimated using blood samples collected in EDTA vials on day 0, 30<sup>th</sup> and 60<sup>th</sup> day of experiment. Serum biochemical parameters like alanine amino transferase (ALT), aspartate amino transferase (AST), total protein and albumin were estimated from the serum samples collected on 0, 30<sup>th</sup> and 60<sup>th</sup> day of experiment using semi automatic biochemical analyser (ARTOS, Bangalore). Egg production (per day/ group) was recorded till the end of experiment. Weekly representative egg samples from each group were analysed for shape index, shell thickness. Eggs were weighed daily till the end of the experiment. At the end of the experiment all birds were sacrificed and necropsy was conducted on each carcass to observe any gross pathological changes. The representative tissue samples were processed for histopathology and stained with haematoxylin and eosin (Luna, 1968).

### Statistical Analysis

Mean values and standard error of means were calculated and expressed as mean SEM. The data was analyzed by two-way ANOVA with Bonferroni post-tests using GraphPad Prism Trial version 5 for Windows, GraphPad Software, San Diego California USA, www.graphpad.com.

## RESULTS

Mycotoxins like aflatoxin B1, B2, G1, G2 and ochratoxin A were identified and quantified in cultured rice and wheat samples (Figure 1). Aflatoxin concentration in cultured rice sample was 826 ppb (Table 2). Ochratoxin A concentration in cultured wheat sample was 8990 ppb (Table 2). The feed material showed the presence of minimum concentration of aflatoxin B1, B2, G2 and ochratoxin A (Table 2). The observed clinical signs were dullness, ruffled feather, depression and occasional diarrhoea in group II birds. Haemoglobin concentration in Group II, III, V, VI was significantly ( $P<0.01$ ) ( $P<0.001$ ) lower than the control group value (Table 3). Packed cell volume in Group II, III, VI, V, VI was significantly ( $P<0.05$ ) ( $P<0.01$ ) ( $P<0.001$ ) lower than the control group value (Table 3). Total erythrocyte count in Group II, III, V, VI was significantly ( $P<0.05$ ) ( $P<0.01$ ) ( $P<0.001$ ) lower than the control group value (Table 3). Alanine amino transferase in Group II, III, IV, V and VI was significantly ( $P<0.001$ ) higher than the control group value (Table 4). Aspartate amino transferase in Group II, III, IV and V was significantly ( $P<0.001$ ) higher than the control group value (Table 4). Serum albumin in Group II, III, IV, V and VI was significantly ( $P<0.001$ ) lower than the control group value (Table 5). Serum total protein in Group II, III, IV, V and VI

was significantly ( $P<0.01$ ) ( $P<0.001$ ) lower than the control group value (Table 5). Average egg weight in Group II was significantly ( $P<0.01$ ) lower than the control group value (Table 6). Average egg production in Group II was significantly ( $P<0.001$ ) lower than the control group value (Table 7). Egg shell thickness and shape index of weekly representative samples from all the groups did not show any significant difference during the experiment (Table 8, 9). At autopsy, none of the treated groups showed any gross pathological in any of the organ except in group II liver was pale and swollen and kidney was congested and swollen. Microscopically liver from toxin fed group revealed congestion and increase in sinusoidal space, separation of hepatic cords, focal infiltration of inflammatory cells in interstitium (Figure 2.2). Kidney revealed congestion and haemorrhage, swollen tubular epithelial cells with granular eosinophilic cytoplasm, vacuolar degeneration, infiltration of inflammatory cells, mild interstitial connective tissue proliferation, desquamation of epithelial cells, complete degeneration and disappearance of glomeruli (Figure 2.4).

**Table 2:** Identification and quantification of different mycotoxin in fermented rice, fermented wheat and feed samples ( $\mu\text{g}/\text{kg}$  or ppb)

| Mycotoxins   | Samples               |                        |                 |
|--------------|-----------------------|------------------------|-----------------|
|              | Fermented Rice Powder | Fermented Wheat Powder | Layer Mash Feed |
| Aflatoxin B1 | 800.00                | 0.83                   | 0.69            |
| Aflatoxin B2 | 21.40                 | 1.95                   | 1.64            |
| Aflatoxin G1 | 5.68                  | 4.90                   | NP              |
| Aflatoxin G2 | 0.66                  | 8.69                   | 0.91            |
| Ochratoxin A | 35.50                 | 8990                   | 1.12            |
| T-2 Toxin    | NP                    | NP                     | NP              |
| HT-2 Toxin   | NP                    | NP                     | NP              |
| DAS          | NP                    | NP                     | NP              |
| DON          | NP                    | NP                     | NP              |
| 3Ac-DON      | NP                    | NP                     | NP              |
| 15-AcDON     | NP                    | NP                     | NP              |
| Fumonisin B1 | NP                    | NP                     | NP              |
| Fumonisin B2 | NP                    | NP                     | NP              |
| Zearalenone  | NP                    | NP                     | NP              |
| Citrinin     | NP                    | NP                     | NP              |
| Beauvericin  | NP                    | NP                     | NP              |
| Apicidin     | NP                    | NP                     | NP              |

NP: No Peak

**Table 3:** Effect of feed treatment with and without toxin and toxin binders on hematological parameters of white leghorn laying hens

| Groups    | Hemoglobin (g%) |                   |                  | Packed Cell Volume (%) |                |                   | Total Erythrocyte Count (millions/ $\mu\text{L}$ ) |                 |                  |
|-----------|-----------------|-------------------|------------------|------------------------|----------------|-------------------|--|-----------------|------------------|
|           | Day 0           | Day 30            | Day 60           | Day 0                  | Day 30         | Day 60            | Day 0  | Day 30          | Day 60           |
| Group I   | 8.3 $\pm$ 0.1   | 9.2 $\pm$ 0.1     | 11.1 $\pm$ 0.3   | 35.7 $\pm$ 0.6         | 30.7 $\pm$ 1.3 | 34.8 $\pm$ 0.8    | 2.5 $\pm$ 0.1                                      | 2.3 $\pm$ 0.1   | 2.1 $\pm$ 0.0    |
| Group II  | 8.3 $\pm$ 0.2   | 8.1 $\pm$ 0.2**   | 8.0 $\pm$ 0.2*** | 33.3 $\pm$ 1.5         | 29.7 $\pm$ 1.3 | 25.0 $\pm$ 1.1*** | 2.6 $\pm$ 0.2                                      | 1.9 $\pm$ 0.1** | 1.6 $\pm$ 0.1*** |
| Group III | 8.4 $\pm$ 0.1   | 9.5 $\pm$ 0.3     | 10.2 $\pm$ 0.2** | 36.0 $\pm$ 0.9         | 33.2 $\pm$ 0.9 | 30.3 $\pm$ 1.6*   | 2.4 $\pm$ 0.1                                      | 2.1 $\pm$ 0.0   | 1.8 $\pm$ 0.1*   |
| Group IV  | 8.3 $\pm$ 0.1   | 9.0 $\pm$ 0.1     | 10.7 $\pm$ 0.1   | 35.8 $\pm$ 0.9         | 33.0 $\pm$ 0.9 | 29.3 $\pm$ 0.7**  | 2.4 $\pm$ 0.1                                      | 2.2 $\pm$ 0.1   | 1.9 $\pm$ 0.1    |
| Group V   | 8.5 $\pm$ 0.2   | 8.4 $\pm$ 0.1**   | 8.3 $\pm$ 0.1*** | 35.7 $\pm$ 1.2         | 28.7 $\pm$ 1.7 | 25.0 $\pm$ 1.3*** | 2.5 $\pm$ 0.1                                      | 2.3 $\pm$ 0.1   | 1.8 $\pm$ 0.1*   |
| Group VI  | 8.3 $\pm$ 0.1   | 11.0 $\pm$ 0.2*** | 9.1 $\pm$ 0.1*** | 34.2 $\pm$ 1.2         | 27.2 $\pm$ 0.5 | 22.3 $\pm$ 0.5*** | 2.4 $\pm$ 0.1                                      | 2.1 $\pm$ 0.0   | 1.8 $\pm$ 0.1*   |

Values are Mean  $\pm$  SE, n=6 \*\*\* $P<0.001$ , \*\* $P<0.01$ , \* $P<0.05$

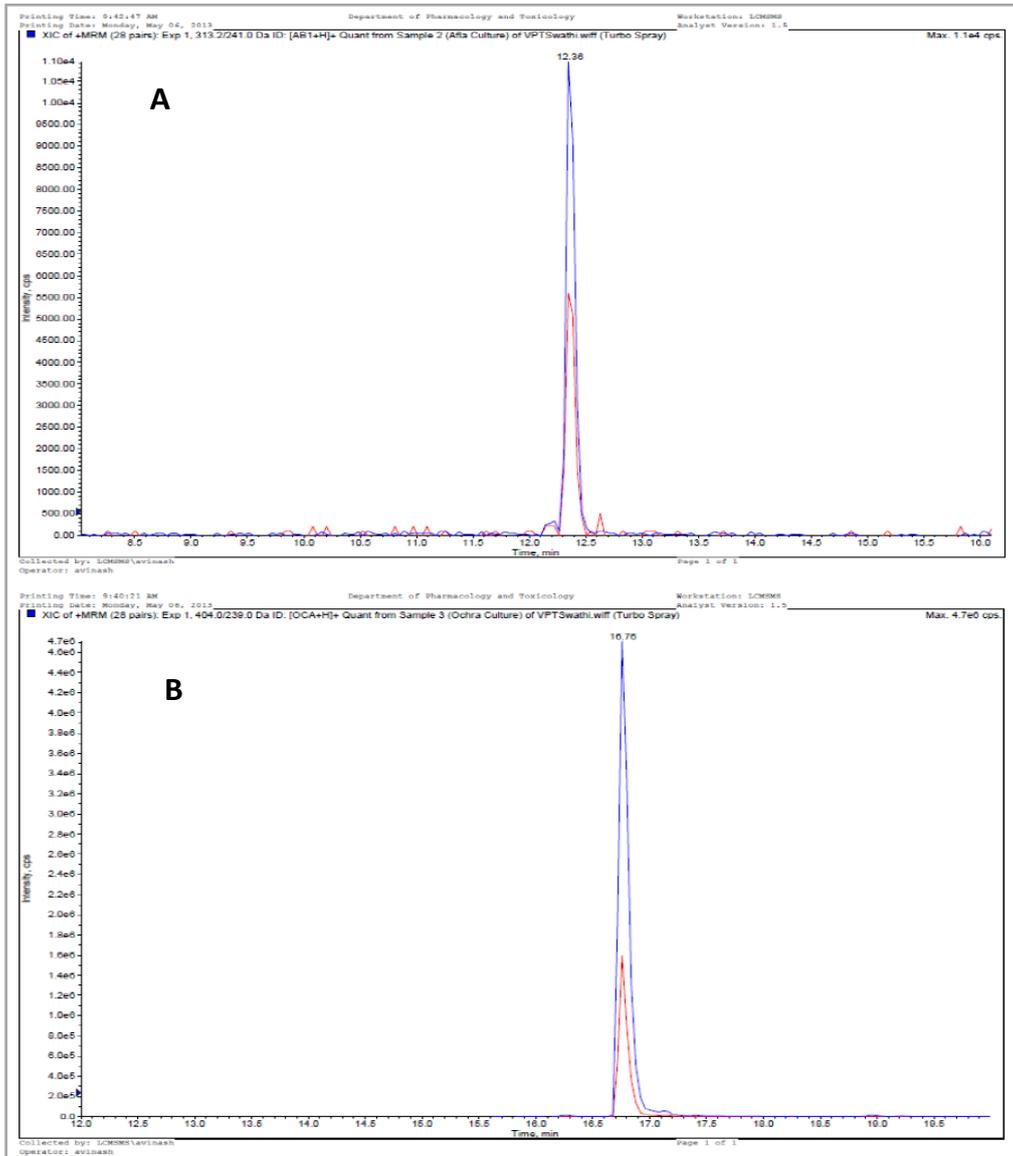


Figure 1: Extracted ion chromatogram for AFB1 (A) and ochratoxin A in rice and wheat sample respectively

Table 4: Effect of feed treatment with and without toxin and toxin binders on Alanine Amino Transferase (ALT) and Aspartate Amino Transferase (AST) level of white leghorn laying hens

| Groups    | ALT ( U/L) |             |             | AST ( U/L) |               |               |
|-----------|------------|-------------|-------------|------------|---------------|---------------|
|           | Day 0      | Day 30      | Day 60      | Day 0      | Day 30        | Day 60        |
| Group I   | 24.2±0.9   | 24.1±0.9    | 23.2±0.5    | 93.2±2.2   | 119.6±1.6     | 124.1±1.2     |
| Group II  | 25.5±1.0   | 38.7±0.8*** | 52.6±2.4*** | 95.3±1.5   | 167.4±10.0*** | 191.9±6.3***  |
| Group III | 24.3±0.5   | 33.0±0.9*** | 43.2±2.0*** | 97.7±1.0   | 134.7±2.2     | 153.9±10.5*** |
| Group IV  | 25.1±0.7   | 34.0±1.3*** | 40.6±1.5*** | 96.5±1.2   | 125.0±1.4     | 151.5±8.0***  |
| Group V   | 23.9±0.4   | 34.2±1.6*** | 41.1±2.5*** | 98.4±0.4   | 122.1±2.2     | 167.5±10.2*** |
| Group VI  | 25.3±0.5   | 35.8±1.1*** | 40.8±1.6*** | 97.2±1.0   | 113.4±4.0     | 138.7±4.1     |

Values are Mean ±SE, n=6 \*\*\*P<0.001, \*\*P<0.01, \*P<0.05

Table 5: Effect of feed treatment with and without toxin and toxin binders on serum albumin and total protein level of white leghorn laying hens

| Groups    | Serum albumin (g/dL) |            |            | Serum total protein (g/dL) |            |            |
|-----------|----------------------|------------|------------|----------------------------|------------|------------|
|           | Day 0                | Day 30     | Day 60     | Day 0                      | Day 30     | Day 60     |
| Group-I   | 3.3±0.1              | 3.3±0.1    | 3.3±0.0    | 5.3±0.1                    | 5.1±0.1    | 5.0±0.2    |
| Group-II  | 3.1±0.1              | 2.4±0.1*** | 2.7±0.1*** | 5.5±0.1                    | 4.3±0.1*** | 3.7±0.2*** |
| Group-III | 3.3±0.1              | 3.0±0.0    | 2.8±0.0*** | 5.2±0.0                    | 4.6±0.1**  | 3.8±0.2*** |
| Group-IV  | 3.4±0.2              | 3.0±0.1    | 2.5±0.1*** | 5.2±0.0                    | 4.5±0.1*** | 4.4±0.2*** |
| Group-V   | 3.2±0.1              | 3.5±0.1    | 2.9±0.1**  | 5.6±0.1                    | 4.6±0.1**  | 4.0±0.1*** |
| Group-VI  | 3.3±0.1              | 3.0±0.1    | 2.2±0.2*** | 5.5±0.1                    | 4.6±0.1**  | 3.7±0.1*** |

Values are Mean ±SE, n=6 \*\*\*P<0.001, \*\*P<0.01, \*P<0.05

**Table 6:** Effect of feed treatment with and without toxin and toxin binders on average egg weight (g) of white leghorn laying hens

| Week | Group      |             |            |            |            |            |
|------|------------|-------------|------------|------------|------------|------------|
|      | I          | II          | III        | IV         | V          | VI         |
| 1    | 54.23±1.31 | 52.66±3.11  | 56.62±2.52 | 52.16±1.37 | 52.66±2.95 | 54.16±1.04 |
| 2    | 51.00±0.89 | 55.00±2.67  | 51.66±2.07 | 50.66±2.91 | 54.66±1.60 | 51.33±2.17 |
| 3    | 48.16±1.93 | 52.16±2.33  | 52.66±2.55 | 53.83±1.99 | 52.66±1.49 | 46.66±2.06 |
| 4    | 53.16±2.34 | 50.16±1.26  | 55.05±0.90 | 48.13±3.45 | 51.41±1.54 | 52.33±1.89 |
| 5    | 48.66±0.84 | 49.86±1.60  | 54.58±1.68 | 51.81±2.03 | 53.08±1.07 | 52.80±1.33 |
| 6    | 53.00±1.00 | 53.90±1.78  | 55.10±1.66 | 53.68±1.63 | 54.50±0.61 | 52.85±1.85 |
| 7    | 52.66±1.25 | 49.71±1.91  | 54.30±1.95 | 54.73±0.83 | 54.81±0.71 | 51.85±1.10 |
| 8    | 51.00±1.52 | 56.25±1.61  | 53.20±1.44 | 53.08±1.62 | 56.38±0.40 | 55.93±1.30 |
| 9    | 55.50±1.20 | 56.10±2.62  | 54.88±1.35 | 54.91±1.09 | 57.75±0.92 | 54.73±1.08 |
| 10   | 58.00±1.36 | 50.71±2.91* | 54.33±2.51 | 56.70±0.85 | 57.95±0.99 | 56.83±1.51 |

Values are Mean ±SE, n=6, \*P&lt;0.05

**Table 7:** Effect of feed treatment with and without toxin and toxin binders on average egg production (per day/group) of white leghorn laying hens

| Week | Group     |              |           |           |           |            |
|------|-----------|--------------|-----------|-----------|-----------|------------|
|      | I         | II           | III       | IV        | V         | VI         |
| 1    | 6.93±0.06 | 6.93±0.06    | 7.00±0.00 | 6.80±0.10 | 7.00±0.00 | 7.00±0.00  |
| 2    | 6.93±0.06 | 6.80±0.10    | 6.53±0.19 | 6.40±0.21 | 6.73±0.15 | 6.40±0.23  |
| 3    | 6.80±0.10 | 6.40±0.21    | 6.60±0.16 | 6.73±0.11 | 6.45±0.23 | 6.60±0.19  |
| 4    | 6.80±0.10 | 6.46±0.19    | 6.60±0.19 | 6.53±0.53 | 6.23±0.23 | 6.66±0.12  |
| 5    | 6.53±0.13 | 6.73±0.11    | 6.20±0.14 | 6.26±0.11 | 6.53±0.16 | 6.33±0.18  |
| 6    | 6.60±0.13 | 6.46±0.16    | 6.60±0.13 | 6.80±0.10 | 6.86±0.14 | 6.93±0.60  |
| 7    | 6.46±0.13 | 6.06±0.11    | 6.66±0.12 | 6.26±0.22 | 6.83±0.16 | 6.46±0.13  |
| 8    | 6.13±0.21 | 5.73±0.18    | 6.73±0.18 | 6.40±0.21 | 6.63±0.13 | 6.13±0.19  |
| 9    | 6.06±0.26 | 5.13±0.09*** | 6.00±0.13 | 6.06±0.20 | 6.53±0.13 | 5.46±0.13  |
| 10   | 6.00±0.30 | 4.66±0.23*** | 6.33±0.18 | 5.73±0.20 | 6.26±0.11 | 5.23±0.11* |

Values are Mean ±SE, n=6 \*\*\*P&lt;0.001, \*\*P&lt;0.01, \*P&lt;0.05

**Table 8:** Effect of feed treatment with and without toxin and toxin binders on shell thickness (mm) of weekly representative samples of white leghorn laying hens

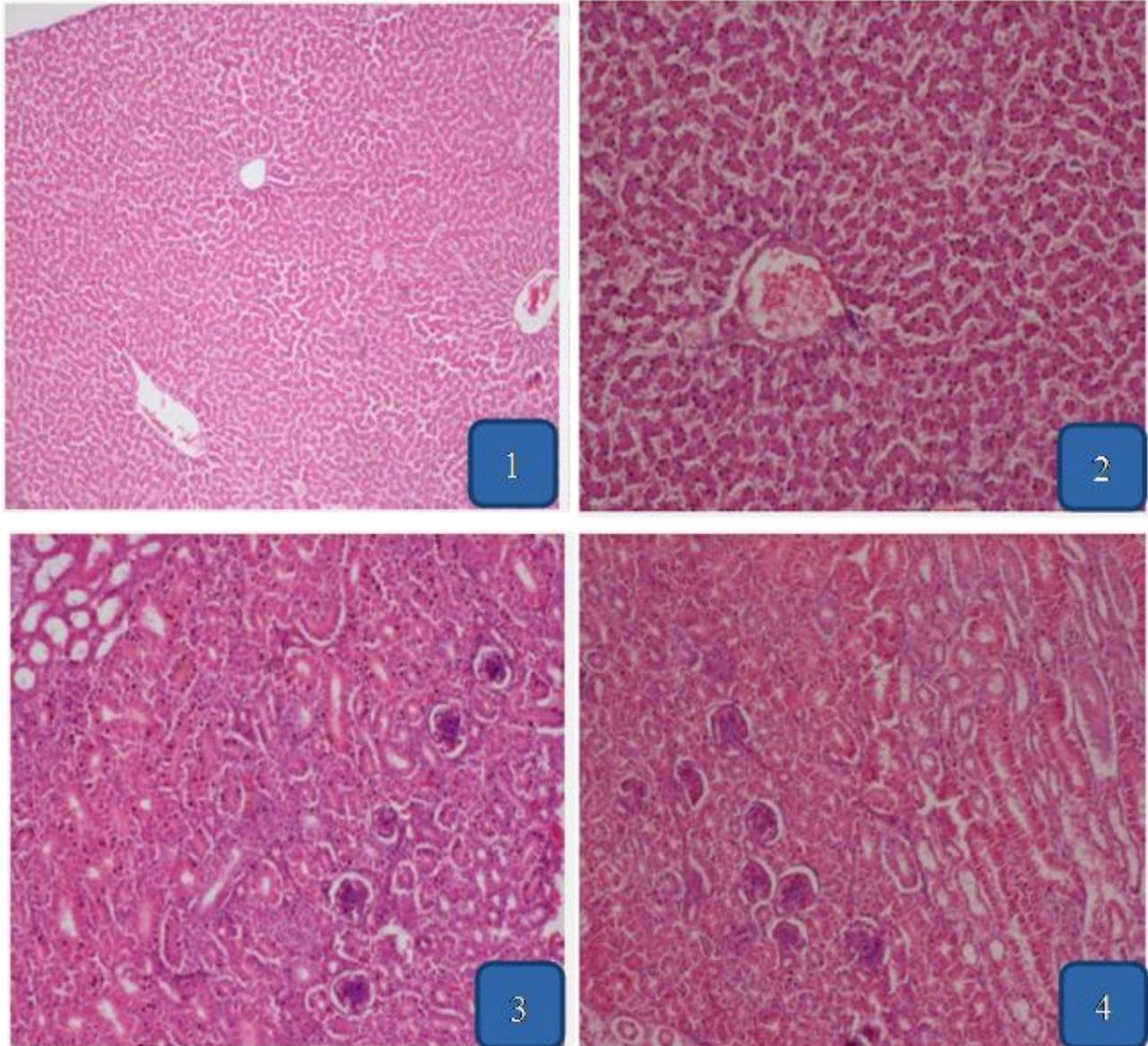
| Week | Group     |           |           |           |           |           |
|------|-----------|-----------|-----------|-----------|-----------|-----------|
|      | I         | II        | III       | IV        | V         | VI        |
| 1    | 0.24±0.02 | 0.18±0.01 | 0.22±0.02 | 0.25±0.04 | 0.21±0.01 | 0.21±0.01 |
| 2    | 0.24±0.04 | 0.21±0.02 | 0.26±0.01 | 0.20±0.02 | 0.26±0.02 | 0.27±0.04 |
| 3    | 0.24±0.02 | 0.20±0.02 | 0.23±0.04 | 0.18±0.01 | 0.19±0.01 | 0.24±0.02 |
| 4    | 0.26±0.03 | 0.18±0.01 | 0.27±0.01 | 0.19±0.0  | 0.23±0.04 | 0.26±0.03 |
| 5    | 0.19±0.02 | 0.21±0.02 | 0.25±0.02 | 0.25±0.02 | 0.25±0.02 | 0.21±0.01 |
| 6    | 0.25±0.02 | 0.21±0.03 | 0.25±0.03 | 0.26±0.01 | 0.23±0.02 | 0.27±0.02 |
| 7    | 0.21±0.01 | 0.25±0.04 | 0.22±0.04 | 0.27±0.04 | 0.18±0.01 | 0.19±0.02 |
| 8    | 0.18±0.01 | 0.25±0.01 | 0.26±0.01 | 0.23±0.01 | 0.18±0.01 | 0.20±0.01 |
| 9    | 0.21±0.02 | 0.22±0.03 | 0.24±0.04 | 0.22±0.04 | 0.25±0.03 | 0.25±0.02 |
| 10   | 0.25±0.02 | 0.19±0.02 | 0.26±0.01 | 0.24±0.01 | 0.18±0.01 | 0.20±0.02 |

Values are Mean ±SE, n=6 \*\*\*P&lt;0.001, \*\*P&lt;0.01, \*P&lt;0.05

**Table 9:** Effect of feed treatment with and without toxin and toxin binders on shape index of weekly representative samples of white leghorn laying hens

| Week | Group      |            |            |            |            |            |
|------|------------|------------|------------|------------|------------|------------|
|      | I          | II         | III        | IV         | V          | VI         |
| 1    | 77.61±0.81 | 77.32±0.31 | 78.53±0.62 | 77.57±1.19 | 78.74±0.56 | 78.17±0.39 |
| 2    | 78.38±1.11 | 76.76±0.47 | 77.22±0.23 | 78.53±0.97 | 77.50±0.50 | 78.28±0.87 |
| 3    | 77.52±1.06 | 78.04±1.03 | 77.90±0.53 | 78.57±0.61 | 76.13±0.63 | 77.22±0.74 |
| 4    | 77.32±0.66 | 77.57±1.19 | 78.74±0.56 | 78.09±0.87 | 77.50±0.50 | 78.28±0.87 |
| 5    | 77.67±0.62 | 78.53±0.97 | 77.50±0.50 | 76.85±0.67 | 77.50±0.50 | 78.28±0.87 |
| 6    | 76.42±0.77 | 78.57±0.61 | 76.13±0.63 | 77.22±0.48 | 77.50±0.75 | 78.82±0.93 |
| 7    | 78.53±0.62 | 77.57±1.19 | 78.74±0.56 | 77.76±0.70 | 77.33±0.68 | 77.64±0.82 |
| 8    | 77.22±0.23 | 78.53±0.97 | 77.50±0.50 | 76.50±0.41 | 78.53±0.97 | 78.61±0.87 |
| 9    | 77.90±0.53 | 78.57±0.61 | 76.13±0.63 | 76.74±0.38 | 76.74±0.38 | 77.17±0.75 |
| 10   | 77.29±0.80 | 79.27±0.54 | 77.24±0.48 | 77.64±0.60 | 76.91±0.57 | 78.85±0.85 |

Values are Mean ±SE, n=6 \*\*\*P&lt;0.001, \*\*P&lt;0.01, \*P&lt;0.05



**Figure 2:** Microscopical sections of liver and kidney of Group I and II white leghorn laying hen, (1) liver section of Group I (2) liver section of Group II (3) kidney section of Group I and (4) kidney section of group II (H and E 10 X)

## DISCUSSION

The mycotoxicated birds showed ruffled feathers, dullness, depression and occasional diarrhoea. Observations are in concomitance with those reported by Sawale *et al.* (2009). The haematological parameters showed significantly decreased level in treated groups (Group II, III, IV, V and VI) compared to their respective control group (Group I). This was in accordance with the findings of Mani *et al.* (1993) and Ramadevi *et al.* (2000) who reported decrease in Hb, TEC and PCV values in broilers fed with aflatoxin and ochratoxin respectively. Improvement in haematological values were recorded in treatment groups supplemented with Vilocym® in group III, Toxiroak® in group IV, Vilocym-z® in V and AV/LBP/20® in VI as compared to the mycotoxin fed group II during the experiment and the values are comparable with control group I fed with basal diet. These results are in correlation with earlier findings of Sawarkar *et al.* (2011) on AF and OA at 100 ppb each in feed and its amelioration with Toxiroak Gold® in broilers. Sawarkar *et al.* (2012) reported that broilers feed with AF and OA at 100 ppb each in feed with Vilocym-Z® in broilers showed

lowered reduction in haematological values. The result indicated that herbal toxin binders could improve the haematological parameters of leghorn hens by binding with toxin in the feed and making toxin less effective.

Serum albumin and serum total protein level significantly decreased in treated groups (Group II, III, IV, V and VI) compared to their respective control group (Group I). This was in accordance with the findings of Umar *et al.* (2012) who reported significant decrease in serum total protein values in broilers fed with aflatoxin and ochratoxin in feed at 3mg/kg each for 6 weeks. Sawale *et al.* (2009) reported significant decrease in total serum protein and albumin in layers fed with ochratoxin A at 1 ppm from 26 weeks age and its amelioration with Toxiroak®.

The biochemical parameters showed significantly decreased level in treated groups (Group II, III, IV, V and VI) compared to their respective control group (Group I). This was in accordance with the findings of (Sawarkar *et al.* 2011 and 2012) who reported decrease in serum

protein concentration and increased ALT and AST level was reported in broilers fed with aflatoxins and ochratoxins at 100ppb each for 42 days and its amelioration with Toxiroak Gold® and Vilocym-Z®. The result suggested that birds feed with herbal toxin binder could lowered biochemical changes in the leghorn laying hens owing to the effect of antioxidative activity of natural phytochemicals in herbal binders.

Egg shell thickness and shape index of weekly representative samples from all the groups did not show any significant difference during the experiment. Egg weight and egg production significantly decreased in Group II compared to control group. This was in accordance with Manafi and Khosravinia, (2013) who reported decreased egg production and egg weight in broiler breeders fed with 500 µg aflatoxin B1/kg feed. This was also in concomitance with the findings of Sawale *et al.* (2009) reported similar results in average egg production in layers fed with ochratoxin at 1 ppm and found improvement with Toxiroak® feed. Histopathology Group II birds revealed mycotoxin effects on liver and kidney. Microscopic lesion on liver are in agreement with report of Sawarkar *et al.* (2011) in broilers fed with aflatoxins and ochratoxins at 100 ppb each and amelioration with Toxiroak Gold®. Microscopic lesions on kidney are in agreement with report of Anitha 2007 in broilers fed with ochratoxins at 1 ppm and amelioration with herbal oil and Toxiroak Gold®. Degenerative change in tubules and glomeruli was also in accordance with the findings of Mohiuddin *et al.* (1992) and Ramadevi *et al.* (1998).

## CONCLUSIONS

Feeding aflatoxin and ochratoxin in combination significantly reduced egg weight, egg production, haematological parameters, total serum proteins, albumin and increased serum ALT and AST levels. In addition mycotoxin fed birds also showed microscopic lesions in liver and kidney, clinical signs of dullness, depression, diarrhoea and ruffled feathers indicating adverse effects on general health of layer chickens. Supplementation of herbal mycotoxin binders in mycotoxicated feed showed improvement in all the above discussed parameters indicating that herbal mycotoxin binders reduce the severity of toxicity. This would be helpful to the farming community to reduce ill effects of mycotoxins. Moreover, herbal toxin binders could be safe and natural source to avoid mycotoxicosis in animal.

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