

# Activity of Pomegranate Peels and Clove Powders in Detoxification of Aflatoxin B1 and Ochratoxin A from Contaminated Poultry Diet

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## Abstract

The study was conducted to evaluate the effect of contaminated poultry diet with AfB1, OTA on chicks feds on this diet and the efficiency of addition pomegranate peels and clove powders in detoxifying the mycotoxins. Results showed that two mycotoxins caused significant reduction in chick's weight with high mortality percentage compared with control (non-contaminated diet). The chick weights were found to be 518.85, 532.90, 418.97 g/chick associated with mortality of 20, 25, 35% for chick fed on diet contaminated with AfB1, OTA, and a combination of each respectively compared with 801.63 g/chick in control. The two mycotoxins caused significant reduction in packed cell volume, hemoglobin concentration, red blood cell and protein concentration, 27.62%, 8.24 g/100 ml,  $2.07 \times 10^6$ /ml, 3.25 g/100 ml, 27.25%, 8.77 g/100 ml,  $2.19 \times 10^6$ /ml, 3.74 g/100 ml, 24.07%, 7.22 g/100 ml,  $1.88 \times 10^6$ /ml, 3.10 g/100 ml in chicks blood feds on diet contaminated with OTA, AfB1, and combination of each respectively compared with 38.55%, 11.56 g/100 ml,  $2.98 \times 10^6$ /ml, 4.50 g/100 ml in control respectively. The amendment of the contaminated diet with 5% pomegranate peels powder and 2% clove powder induced significant increases in chicks weight that attained to 740.30, 730.25, 680.50 g/chick, 730.25, 725.00, 675.25 g/chick of chicks feds on diet contaminated with AfB1, OTA, and combination of each respectively compared with 535.90, 518.85, 418.79 g/chick in control respectively associated with high reduction in mortality, 5% compared with 20, 25, 35% in control respectively.

**Keywords:** AfB1; Detoxification; Medicinal plant powders; OTA; Poultry diet

## Introduction

The contamination of poultry diet with fungi producing mycotoxins is considered as one of the most important problems confronting poultry breeders worldwide [1]. Corn seeds, the main constituent of poultry diet, has been reported to be infected with many fungi producing mycotoxins in the field and in storage and considered the main source of diet contamination with mycotoxins [2,3]. Of the fungi found associated with poultry diet and producing mycotoxin, *Aspergillus flavus* producing aflatoxin B1, and *A. ochraceus* producing ochratoxin A were the more prevalent.

Both mycotoxin caused enormous problems to both chicks feds on contaminated diet and to human consumed meat of chicken previously feds on such diet [4-6]. The two mycotoxins reported to cause renal failure, hydronephrosis and chlorosis of kidney, necrosis in liver cells, anemia and reduction in blood components in birds feds on contaminated diet [7,8]. The problem became more complicated in case of diet contaminated with more than one mycotoxin that exert synergistic effect [9-12]. A preliminary study at our department showed that poultry diet is highly contaminated with AfB1 producing by *Aspergillus flavus* and ochratoxin A producing by *A. ochraceus* that may be introduced with corn seeds, main part in the diet, previously infected in the field or contaminated and developed under storage condition [13].

The study was conducted to evaluate the effect of double contamination of poultry diet with aflatoxin B1 and ochratoxin A on chicks and the efficiency of pomegranate pericarps and clove powders in detoxification the mycotoxins from the diet.

## Materials and Methods

### Fungal isolates

The fungal isolates were isolated from poultry diet collected from

different locations in Iraq on potato dextrose agar (PDA) in petriplates of 9 cm diameter. The growing fungi were purified and identified as describe by Klich and Pitt [14].

### Test of isolates ability to produce mycotoxin

The isolates of *A. flavus* and *ochraceus* were cultivated on rice seeds for producing mycotoxin. Hundred ml distilled water were added to 150 g rice seeds in 250 ml flasks, the flasks were autoclaved at 121°C and 1.5 kg/cm<sup>2</sup> for 20 min in two successive days. Two discs, 0.5 cm diameter, from fungal colony 7 days old were added into each flask. The flasks were agitated for homogenization and incubated at  $25 \pm 2^\circ\text{C}$  for 21 days [13]. The contaminated seeds were oven dried at 50°C in paper sacs and ground to fine powder.

### Mycotoxin extraction and detection

A sample of contaminated rice seeds powder was used for mycotoxin extraction as described by Hussein et al. [15]. The extracted mycotoxin was identified by thin layer chromatography on plates of silica gel G60, 20 × 20 × 0.25 cm using standard AfB1, OTA (sigma chemical co.) as control as described by Asonso et al. [16].

### Evaluation of mycotoxin concentration

The mycotoxin concentration was estimated by High Performance

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Liquid Chromatography (HPLC) system, model LC 20/OA, shimadzu co. Koyoto, Japan, in reverse phase column C18 DB (50 × 4.6 mm) 3 mm particle size with mobile phase 0.01 N potassium phosphate solution (KH<sub>2</sub>PO<sub>4</sub>) PH 6.0 at flow rate 1 ml/min. The absorbance values were followed by spectrophotometer at 220 nm, and the concentration of the mycotoxin was evaluated by comparison the absorption curve obtained with mycotoxin standard curve by the following equation:

Mycotoxin concentration=area of sample curve/area of mycotoxin× standard conc. × dilution factor [17].

### Plant powders

**Note:** Pomegranate peel and clove powders were purchased from local commercial market.

**Activity of medicinal plant powders in mycotoxin detoxification:** A poultry diet composed of, 39% corn seeds, 30% wheat seeds, 20% soybean, 10% concentrated protein, 0.7 calcium, 0.3% NaCl was used in this experiment. Powders of rice seeds containing aflatoxin B1 and ochratoxin A were mixed separately and in combination with the diet to obtain final concentration 2 ppm of each mycotoxin. The diet was homogenized with water to obtain relative humidity 15%. A part of each diet was amended with 5% pomegranate peel powder and the other with 2% clove powder, chicks of 51 g each (2 days old) were left to feed on the contaminated diet and other chicks on mycotoxin free diet as control The chicks were distributed in Complete Randomized Design (CRD) with 10 treatments and 3 replications, 6 chicks/replication as following:

T<sub>1</sub>=diet contaminated with Afb1.

T<sub>2</sub>=diet contaminated with Afb1+5% pomegranate peels powder.

T<sub>3</sub>=diet contaminated with Afb1+2% clove powder.

T<sub>4</sub>=diet contaminated with OTA.

T<sub>5</sub>=diet contaminated with OTA+5% pomegranate peels powder.

T<sub>6</sub>=diet contaminated with OTA+2% clove powder.

T<sub>7</sub>=diet contaminated with Ochra A, Afla B1.

T<sub>8</sub>=diet contaminated with Ochra A, Afla B1+ 5% pomegranate peels powder.

T<sub>9</sub>=diet contaminated with Ochra A, Afla B1+ 2% clove powder.

T<sub>10</sub>=mycotoxin free diet (control).

The chicks weight were estimated weekly for 4 weeks and the mortality percentage was calculated by the equation is

% mortality=No. of death chicks/total number of chicks × 100.

After 30 days of feeding on the diet, 6 chicks of each treatment were bled and the blood was collected in tubes containing anticoagulant (potassium EDTA and sodium citrate). Packed Cell Volume (PCV), Red Blood Cell (RBC) number, White Blood Cell (WBC) number,

hemoglobin concentration and total protein concentration in the blood were calculated.

## Results

### Fungal isolates

Of several fungi isolated from contaminated poultry diet, *Aspergillus flavus* and *A. ochraceus* isolates were found to be the more prevalent. The majority of the isolates obtained were able to produce mycotoxin as proved by Thin Layer Chromatography (TLC) on silica gel plates. The more active isolate of *A. flavus* producing Afb1, and that of *A. ochraceus* producing OTA as determined by High Performance Liquid Chromatography (HPLC) system were used in this study.

### Effect of Afla B1, Ochra A in the poultry diet on chicks weight and mortality

Results in Table 1 showed that feeding chicks on diet containing Afb1 , OTA, and combination of both at 2 ppm each caused significant reduction in chicks weight associated with high percentage of mortality compared with control (mycotoxin free diet). The mean of chicks weight feds on diet contaminated with Afb1 , OTA, and a combination of the two toxins were attained to 518.82, 532.90, 418.79 g/chick respectively compared with 801.63 g/chick in control. The reductions in chick weight were found associated with 25, 20, and 35% mortality percentage for the three treatments respectively compared with 0.00% in control.

### Effect of Afb1, OTA in the diet on chicks blood components

Results in Table 2 showed that feeding chicks on diet contaminated with Afb1 , OTA has led to significant reduction in Packed Cell Volume (PCV), Hemoglobin Concentration (HC) and Red Blood Cell (RBC) associated with significant increase in White Blood Cell (WBC) compared with control. PCV, Hb, RBC, WBC number were found to be 27.62%, 8.24 g/100 ml, 2.07 × 10<sup>6</sup>/ml, and 30.11 × 10<sup>3</sup>/ml respectively of chicks blood fed on diet containing OTA, 27.25%, 8.77 g/100 ml, 2.19 × 10<sup>6</sup>/ml, 28.98 × 10<sup>3</sup>/ml respectively of chicks blood feds on diet containing Afb1, 24.07%, 7.22 g/100 ml, 1.88 × 10<sup>6</sup>/ml, 35.97 × 10<sup>3</sup>/ml respectively of chicks blood feds on diet containing a combination of the two mycotoxin compared with 38.55%, 11.56 g/100 ml, 2.98 × 10<sup>6</sup>/ml, 23.13 × 10<sup>3</sup>/ml respectively in control. The variation in blood components was found associated with significant decrease of total protein in the blood 3.25, 3.74, 3.10, g/100 ml in chicks blood feds on diet contaminated with Afb1, OTA, combination of the two mycotoxins respectively compared with 4.5 g/100 ml in control.

### Effect of medicinal plant powders in the diet on chicks weight and mortality

Result in Table 3 showed that addition of pomegranate peels powder at 5% and clove powder at 2% into poultry diet induced significant increase in chicks weight compared with control (diet contaminated with mycotoxin non-treated with plant powders). The weight of chicks feeding on diet contaminated with Afb1, OTA, and

Treatment	1 <sup>st</sup> week	2 <sup>nd</sup> week	3 <sup>rd</sup> week	4 <sup>th</sup> week	% Mortality
Diet contaminated with OTA	102.51	198.80	375.80	518.82	25
Diet contaminated with Afb1	104.67	208.57	389.57	532.90	20
Diet contaminated with OTA + Afb1	93.87	150.00	204.22	418.97	35
Non-contaminated Diet (control)	129.82	312.27	524.43	801.63	0
LSD=0.05	5.11	16.71	60.21	72.1	

**Table 1:** Effect of feeding chicks on diet contamination with Afb1, OTA on chicks weight and mortality.

Treatment	% PVC	Hb concentration	Red blood cells number (RBC) 10 <sup>6</sup> /ml	white blood cells number (WBC) 10 <sup>3</sup> /ml	Total protein g/ml
Diet contaminated with OTA	27.62	8.24	2.07	30.11	3.25
Diet contaminated with AFB1	27.25	8.77	2.19	28.98	3.74
Diet contaminated with OTA+AFB1	24.07	7.22	1.88	35.97	3.10
Non-contaminated Diet (control)	38.55	11.56	2.98	23.13	4.50
LSD=0.05	2.43	0.62	0.57	3.11	0.89

Table 2: Effect of feeding chicks on diet contaminated with AFB1, OTA on blood components.

Treatment	Chick/weight/g	Mortality percentage
T1	532.90	20
T2	740.30	5
T3	730.25	5
T4	518.83	25
T5	730.25	5
T6	725.00	5
T7	418.97	35
T8	680.50	6
T9	675.25	5
T10	822.40	0
LSD=0.05	45.61	

Table 3: Effect of amendment of poultry diet with clove and pomegranate powder on chicks weight and mortality.

a combination of the two mycotoxin, and amended with pomegranate peels and clove powders were found to be 740.30, 730.25, 680.5 g/chick, 730.25, 725.00, 675.25 g/chick respectively, compared with 532.90, 518.83, and 418.97 g/chick in control respectively, associated with high reduction in mortality percentage, 5% for all the treatment compared with 20, 25, 35% respectively in control.

## Discussion

Result of this study demonstrated that the mycotoxin AFB1, OTA exerted high reduction in chicks weight and in blood components with high mortality percentage. The reduction of chicks weight may be due to the toxic effects of the mycotoxins on different organelles in the cell leading to refuse the diet by the chicks.

The toxic effect of the mycotoxin could be attributed to the ability of the mycotoxin to form a complex with DNA that leading to inhibit DNA replication and transcription and reduce protein synthesis. Several previous studies reported that mycotoxin interact with nucleic acid in liver cells causing modification in DNA structure, disturbance in liver function and liver toxicosis [18-20]. McMasters and Vindani [21], Awaad et al. [22], reported that mycotoxin interact with phenylalanyl-tRNA-synthetase causing reduction in protein synthesis. Other studies reported that OTA caused renal failure with hydronephrosis, decrease in weight, reduction in red blood cells, decrease hemoglobin concentration, and reduction of total protein synthesis in chicken [10,23].

Our results revealed that feeding chicks on diet, contaminated with AFB1, OTA, amended with pomegranate peels and clove powders induce significant increase in chicks weight compared with chicks fed on contaminated non-treated diet. The increase of chicks weight may be attributed to the ability of certain compounds in the medicinal plant powders to combine with the toxin forming complex non-absorbable by the chick cells and get out of the body. It is possible also that the mycotoxins in the diet were degraded by some compounds in the powder through interaction with active groups in the mycotoxin causing breaks in the active ring of the mycotoxin leading to destroy the mycotoxin or convert it to non-toxic compounds.

Several studies reported that medicinal plant extracts contain many active compound including phenolic compounds, organic acids, steroids, triterpens and volatile oil [24-26]. Powders and extracts of many medicinal plants were found inhibit the growth of *A. flavus* and aflatoxin production [27,28]. Other studies reported that some herbal extract have the potential to degrade AFB1 [8,28,29]. Velazhahan et al. [30,31] reported the detoxification of AFB1 by seed extract of *Trachyspermum ammi*.

Natural medicinal plant products may provide alternative way, safer and more effective than synthetic chemical to prevent fungal growth and degrading mycotoxins produced in poultry diet.

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