Effects of Prolonged Dietary Exposure to Cadmium on some Hematological and Immunological Parameters of Japanese Quail and Possible Protective Effects of Ascorbic Acid and Garlic

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ABSTRACT: This study was carried out on 200, one-week old unsexed growing Japanese quail chicks to evaluate the toxic effects of cadmium on hematological and immunological parameters and their modulation with certain antioxidants in growing Japanese quail. The quail were divided into five equal groups with forty chicks in each group and each sub group was allotted into four replicates (10 each) in a completely randomized design. Group one fed basal diet only without supplementation (served as control), group 2, fed basal diet + 40 mg cadmium chloride/kg diet, groups 3, 4 and 5 fed basal diet + 40 mg cadmium chloride/kg diet and supplemented with either of 200 mg ascorbic acid / kg diet, 500 mg dried garlic powder /kg diet or 200 mg ascorbic acid / kg diet +500 mg dried garlic powder /kg diet, respectively. Blood samples were collected for biochemical analysis at the end of experiment. Cadmium caused significant ($P \leq 0.01$) decreases in the red blood cells (RBC) counts and hemoglobin (HGB), Packed cell volume (PCV) and numerical decrease hemoglobin (HGB), respectively, as compared with those of the control. While, a no significant increase in white blood cells (WBCs) counts was detected. Combined treatment of cadmium exposed quail with ascorbic acid or/and dried garlic powder had significantly ($P \leq 0.01$) improved RBCs and HGB, however, it not compares favorably with those obtained in the control group. Meanwhile, WBC and PCV were insignificantly affected by the feed additives used in the present study. On the other hand, serum IgG level was significantly decreased and numerical decrease in IgM in cadmium group. Combined treatment of cadmium exposed quail with ascorbic acid or/and dried garlic powder had improved immunity. These results mean that dietary supplementation with ascorbic acid or/and dried garlic powder might be useful in reversing the decrease IgG and IgM induced by cadmium and alleviated the adverse effect of cadmium on immunity.

Key words: Japanese quail, cadmium, ascorbic acid garlic, hematology, immunity

INTRODUCTION

The diverse deleterious health effect upon exposure to toxic heavy metals in the environment is a matter of serious concern and a global issue (Patra *et al.* 2011). It was established that, environmental pollutants (heavy metals) can produce adverse health effects in human and animals. Such effects are usually in chronic form due to their cumulative property after long time of exposure (Lippmann, 2009). The heavy metals as lead, cadmium, zinc and mercury which are widely used in manufacturing of many industrial and agricultural compounds are considered as a source of pollution to animal and bird diets or drinking water (Adham *et al.*, 2001).

Cadmium (Cd) is a one of the major occupational and environmental pollutants. Animals exposure to Cd occurs chiefly through inhalation or ingestion. Extensive mining and indiscriminate industrialization have increased cadmium contamination of environment. Plants readily absorb cadmium from the soil and accumulate it in various parts of the plant (Bingham *et al*.,1975). Shellfish such as mussels, scallops and oysters and other fish accumulate cadmium and may become a major source of cadmium exposure for poultry and other livestock fed with fish meal and oyster shell grid as calcium source (Alisauskas *et al.,* 2007; Krishnakumar and Bhat, 2006). Cadmium is considerably toxic with destructive impacts on most organ systems such as respiratory, digestive, reproductive, skeletal and cardiovascular systems and some sensitive organs, including liver and kidney (Jama *et al*., 2013). Cd acts as a stimulator for formation of Reactive Oxygen Species (ROS), hydrogen peroxide and hydroxyl radicals. These free radicals enhanced lipid peroxidation, caused oxidative stress, DNA damage, altered calcium and sulfhydryl homeostasis (Sevcikova *et al*., 2011) which adversely affects the performance, hematology and serum biochemical parameters, besides damaging kidney, liver and bursa of fabricius. Antioxidants are substances that protect cells against the adverse effects of xenobiotics, toxicants, drugs and carcinogens. The interest in natural antioxidants, especially of plant origin, has greatly increased in the recent years (Akter *et al.,* 2008; Zeweil *et al.,* 2013) and have been utilized in a prophylactic manner against toxic substances that induced oxidative stress (Aboubakr *et al*., 2014).

Hence, the present study was conducted to evaluate the role of ascorbic acid and garlic powder on hematological and immunological parameters and their modulation in growing Japanese quail fed diets polluted by cadmium.

MATERIALS AND METHODS

Two hundred, one-week old unsexed growing Japanese quail chicks were divided randomly into five groups with forty chicks in each group and each sub group was allotted into four replicates (10 each) in a complete randomized design. The birds were wing -banded, weighted and randomly housed in cages. The house temperature was kept at about 35°C during the first 3days, then gradually decreased by 2 ° C weekly until reached 24° C and kept until the end of the experimental period. In all the experiment groups, the birds were subjected to 23 hours light at intensity of 3 watt / m^2 along the experiment period which extended to the age of 6 weeks, feed and water were available *ad libitum* throughout the experimental period. The basal experimental diet was formulated to cover the nutrient requirements of growing Japanese quail as recommended by NRC (1994). The composition and calculated analysis of the experimental basal diets are presented in Table (1). Each experimental group received one of the following dietary treatments

through the growing period (1 to 6 weeks of age). The order of dietary treatments was as follows:

1. Basal diet only without supplementation (served as control) (T1).
2. Basal diet + 40 mg cadmium chloride/kg diet (T2).
3. Basal diet + 40 mg cadmium chloride/kg diet + 200 mg vitamin C/kg diet.
4. Basal diet + 40 mg cadmium chloride/kg diet + 500 mg dried garlic powder /kg diet.
5. Basal diet +40 mg cadmium chloride/kg diet + vitamin C /kg diet 200 mg +500 dried garlic powder.

Table (1). Composition and calculated analysis of the basal experimental diet

Ingredients	%
Yellow corn	53.30
Soybean meal (44 %)	33.00
Concentrate (50 %) *	10.00
Di-calcium phosphate	0.20
Limestone	1.70
Sunflower oil	0.80
Vit. and min. mix.**	0.50
Salt (NaCl)	0.50
Total	**100**
Calculated analyses[1]:	
Crude protein, %	24.05
ME (Kcal/ kg diet)	2907.10
Ether extract, %	2.44
Crude fiber, %	3.63
Methionine, %	0.76
Methionine + cystine, %	0.88
Lysine, %	1.42
Calcium, %	1.11
Av. Phosphorus	0.39

* Concentrate: ME (K cal/kg) 2870, Crude protein 50%, Crude fiber 1.51%, Crude fat 1.54%, Calcium 4.29%, Phosphorus 2.39 %, NaCl 0.8%, Methionine 4.6%, Methionine & Cystine 5.38%, Lysine 3.90%.

** Each kg of vitamin and minerals mixture contained: Vit. A, 4,000,000 IU; Vit. D_3, 500,000 IU; Vit, E, 16.7 g., Vit. K, 0.67 g., Vit. B1, 0.67 g., Vit. B2, 2 g., Vit. B 6, .67 g., Vit. B12, 0.004 g., Nicotinic acid, 16.7 g., Pantothenic acid, 6.67 g., Biotin, 0.07 g., Folic acid, 1.67 g., Choline chloride, 400 g., Zn, 23.3 g., Mn, 10 g., Fe, 25 g., Cu,1.67 g., I, 0.25 g.,Se, 0.033 g. and,Mg, 133.4 g.

[1] According to NRC (1994).

Vitamin C was obtained from Nasr Pharmaceutical Chemicals Co, Egypt. Dried garlic powder was purchased from National Food LID 12/CL-6, Claremont Road, Civil Lines,75760 Karachi, Pakistan.

Individual blood samples were taken from 3 birds within each treatment (on individual basis) at 6 weeks of age to determine the different hematological parameters. Blood samples were collected on heparin as anticoagulant (0.1 ml of heparin to 1 ml of blood) according to Hawk *et al.* (1965) to be used to determine the total leukocyte count (WBC) according to Natt and Herrick (1952). Blood smears were made and stained for differential leukocyte count (Cook, 1959). White blood cells were counted using magnification count on an AO bright line hemocytometer using light microscope at 100 X. Blood samples were diluted 20 times with a diluted fluid (3 ml acetic acid glacial + 97 ml distilled water + some of Leshman stain) according to Hepler (1966),Hawkey and Dennett (1989). For differential leucocytic count: blood films were prepared from collected blood samples according to the method described by Lucky (1977). A drop of heparinized blood was spread on a glass slide, quickly air dried, fixed by methyl alcohol for 3- 5 min. and stained with Giemsa's stain for 20 minutes, then rinsed under slow water current and staffed gently between two filter paper then examined using oil immersion lens. The percentage of each type of cells was calculated according to Schalm *et al.* (1986). Red blood cells were counted on bright line hemocytometer using light microscope at 400 X magnification. R.B.C's were counted according to the method of Hawkey and Dennett (1989). Hemoglobin concentration was determined of fresh blood samples using hemoglobinometer as the method described by Tietz (1982). Packed cell volume (PCV) was determined according to Schalm *et al.* (1975) by microhaematocrit tubes which were filled approximately to two-thirds full with non-coagulated blood, sealed from one end by special clay and centrifuged at 12000 rpm for 5 minutes. The percentage of packed cells to total volume was determined by direct measurement in a special chart. Serum IgG and IgM were determined using ELISA technique according to the method described by Siwicki and Anderson (1993). The differences among treatments were statistically analyzed by one-way ANOVA using SPSS® (2001) statistical software package for windows version 11.0. The significant differences between treatment means were separated by Duncan's Multiple Range-test (Duncan, 1955).

RESULTS AND DISCUSSIONS

Hematological profile in animals is an important indicator of physiological or pathophysiological status of the body (Khan and Zafar, 2005). Exposure to heavy metals can cause alterations and damage to the hematological profile and hematopoietic system in man and animals (Costa *et al.,* 2004). Results in Table (2) shows that cadmium caused significant ($P \leq 0.01$and 0.001) decreases in the red blood cells (RBC) counts and hemoglobin (HGB) and numerical decrease in Packed cell volume (PCV**)** by 36.1, 18.6 and 8.7% respectively, as compare with those of the control free of cadmium supplementation, respectively. While, a non-significant increase in white blood cells (WBCs) counts was detected. The results presented in Table (3) shows that the percentage of lymphocytes, Heterophils, monocytes and H/L ratio

were not affected by cadmium or by different feed additives as compared to the control group, except eosinophils which were significantly (P ≤ 0.05) increased in cadmium intoxicated groups. While, the additives hadn't the ability to hold back the toxic effect of cadmium on eosinophils. However, these hematological data of the present study indicate that macrocytic hyperchromic anemia has developed in quail treated by cadmium. The same results have been demonstrated by Al-Hamdany (2010) who reported that a significant increase in WBC counts in rats exposed to cadmium chloride due to inflammation and increase stimulate production, but found a significant decrease in Hb resulted by accumulation metal inside the red cell and may be inhibition ferrochelatase enzyme which responsible for linked iron to the globin protein. Also, Ekanem *et al.* (2015) and Sharaf *et al.* (2017) reported that the increased count of white blood cells in rats treated with heavy metals may be due to the inflammatory response induced as defense mechanism. The results presented by Szilagyi *et al.* (1994) showed significantly decreased values of WBC and RBC in chicken

Table (2). Effect of dietary vitamin C and garlic powder on hematological parameters of Japanese quail exposed to cadmium toxicity at 6 weeks of age

Treatments	Hematological Parameters			
	WBCs (10^3/mm^3)	RBCs (10^6/mm^3)	HGB (g/dL)	PCV Value
Control (T1)	16.08	3.93^{a}	11.03^{a}	45.97
Control + Cd (T2)	17.88	2.51^{b}	8.97^{c}	41.97
Control + Cd + Vit C	16.49	3.35^{a}	9.80^{b}	43.57
Control + Cd + Garlic	16.61	3.22^{ab}	10.53^{a}	42.50
Control + Cd + Vit C+ Garlic	16.43	3.84^{a}	10.83^{a}	42.27
SEM	0.230	0.160	0.200	0.790
P value	0.110	0.010	0.010	0.530

Cd: Cadmium chloride supplemented with 40 mg /kg diet, Vit C: Vitamin C supplemented with 200 mg /kg diet, garlic supplemented with 500 mg /kg diet.
$^{a-c}$ Means in the same column having different letters are significantly different (P≤0.05).

Following a longer (6 weeks) load of higher (100 mg/kg) cadmium concentration, Abdo and Abdulla (2011) showed that the hemoglobin amount, hematocrit value, and the total erythrocyte (RBC) count were significantly (P ≤ 0.05) decreased in the blood of treated chicken given a drinking water contained the concentration of 10 mg cadmium /L daily for a period of 30 days. Wintrobe (1978) showed that the reduction of hematological parameters in cadmium treated chicken might be due to the destruction of mature RBCs and the inhibition of erythrocyte production which due to reduction of hemsynthesis that was affected by pollutants. Khangarot and Tripathi (1991) suggested that the decrease in RBCs count may be attributed to hematopathology or acute hemolytic crisis that results in severe anemia in most vertebrates including

chicken species exposed to different environmental pollutants. In addition, James *et al.* (1992) demonstrated that the decrease in the RBCs may be attributed to reduction of growth and other food utilization parameters which results in severe anemia. Moreover, El-Sharkawy and El-Nisr (2012) suggested that cadmium may inhibit heme synthesis by decreasing the absorption of iron from the gastrointestinal tract.

Table (3). Effect of dietary vitamin C and garlic powder on differential white blood cells of Japanese quail exposed to cadmium toxicity at 6 weeks of age

Treatments	Differential white blood cells			
	Lymphocytes (%)	Monocytes (%)	Eosinophils (%)	Heterophils (%)
Control (T1)	61.00	5.33	3.00[b]	30.67
Control + Cd (T2)	60.67	4.67	4.67[a]	30.00
Control + Cd + Vit C	62.00	4.33	3.00[b]	30.67
Control + Cd + Garlic	62.00	6.67	3.67[ab]	27.67
Control + Cd + Vit C+ Garlic	61.00	4.33	2.67[b]	32.00
SEM	1.87	0.330	0.240	1.660
P Value	1.00	0.110	0.045	0.960

Cd: Cadmium chloride supplemented with 40 mg /kg diet, Vit C: Vitamin C supplemented with 200 mg /kg diet, garlic supplemented with 500 mg /kg diet.
[a-b] Means in the same column having different letters are significantly different (P≤0.05).

Recently Andjelkovic *et al.* (2019) found that higher doses of Cd produced a significant decreased in WBC, RBC, PCV and HGB as compared with control group. Our results are in agreement with other researchers using different animal models, route of exposure, and dose regimes, who also observed RBC, HGB, and HCT reductions (El-Boshy *et al.,* 2017, Abdou and Hassan*,* 2014, Mladenovi´c *et al.,* 2014, Sharma *et al.*, 2010, Sharma *et al.,* 2011).

In our study, RBC and HGB were significantly improved by adding the ascorbic acid or/and dried garlic powder, however, it not compares favorably with those obtained in control group. On the other hand, WBC and PCV were insignificantly affected by the feed additives used in the present study. In rats exposed to psychologically stressful situations, aged garlic extracts significantly prevented the decreases in spleen weight seen in control animals. Additionally, the garlic significantly prevented the reduction of hemolytic plaque-forming-cells in spleen cells and anti-SRBC antibody titer in serum caused by this psychological stress. Moreover, a reduction in NK activities was observed in the psychological stressexposed mice as compared with normal mice (non-stress), whereas NK activities in the garlic administered mice were almost equivalent to the mice not exposed to stressors. Garlic was able to block the lipopolysaccaride induced immune cytokine and plasma corticosterone and catecholamine changes following cold water immersion

stress (Nance *et al.,*2006). Aged garlic extract is also effective to prevent adrenal hypertrophy, hyperglycemia and elevation of corticosterone in hyperglycemic mice induced by immobilization stress. Given the extreme chronic stress many people now face during daily life, garlic may prove useful to counter the negative impact this stress has on human physiology.

Measures of immunity that have been commonly used and assessed in poultry are lymphoid organs weights (Pope, 1991), and antibody response to foreign antigens (Klasing, 1998). Lymphoid organs weights are easily measured and reflect the body's ability to provide lymphoid cells during an immune response (Heckert *et al*., 2002). Results on the effect of cadmium and cadmium plus feed additives on IgG and IgM are presented in Table (4). It was observed a significant (P ≤ 0.001) decrease in IgG and numerical decrease in IgM due to intoxicated cadmium as compare with control. Addition of ascorbic acid and dried garlic powder in cadmium diets intoxicated quail resulted in a significant (P ≤ 0.001) improve in the values of IgG and numerical increase in IgM. These results mean that dietary supplementation by ascorbic acid and dried garlic powder might be useful in reversing the decrease in serum IgG and IgM which induced by cadmium and alleviating the adverse effect of cadmium on immunity.

Hassan *et al.* (2012) revealed a decrease in the values of antibody titer due to cadmium chloride groups at different times of the experiment. The least values of antibody titer recorded by animals receiving cadmium chloride alone. The results agree with Ohsawa *et al.* (1988) who reported that when mice were primed with sheep red blood cells after exposure to cadmium chloride, a significant suppression of the antibody forming response was observed in animals fed 300 ppm cadmium chloride, but not in those fed 3 ppm of the same salt. Daum *et al.* (1993) mentioned that cadmium chloride exerted an early inhibitory effect on B- cell activation. This was attributed to the inhibition of RNA, DNA and antibody synthesis. However, selective effects

Table (4). Effect of dietary vitamin C and garlic powder on immunity parameters of Japanese quail exposed to cadmium toxicity at 6 weeks of age

Treatments	Immunity Parameters	
	IgG (mg/ dL)	IgM (mg/ dL)
Control (T1)	261.73[a]	16.57
Control + Cd (T2)	232.70[b]	14.80
Control + Cd + Vit C	236.57[b]	13.97
Control + Cd + Garlic	232.10[b]	14.00
Control + Cd + Vit C+ Garlic	235.23[b]	14.13
SEM	2.76	0.650
P Value	0.001	0.720

Cd: Cadmium chloride supplemented with 40 mg /kg diet, Vit C: Vitamin C supplemented with 200 mg /kg diet, garlic supplemented with 500 mg /kg diet.

[a-c] Means in the same column having different letters are significantly different (P≤0.05).

On the production of specific Ig isotypes by these metals may influence the ability of B-cells to mount effective immune responses to pathogens. Cadmium has been shown to inhibit B-cell cycle entry and humoral immunity.

By measuring the hemagglutination titer and delayed type hypersensitivity response, the results of Lall and Dan (1999) indicated the involvement of adrenal hormones in cadmium induced immunosuppression suggesting that cadmium activates the corticosteroid associated immunoregulatory circuit. Cadmium -administration of cod liver oil in the study of Hassan *et al.* (2012) improved the immune status at different times of the experiment. At the 9th week post-pollutants administration, the viability of lymphocytes was reduced as compared with the control group and the least value was observed in cadmium chloride group. Antioxidants help reduce the oxidizing effect of the pollutants and act as conjugators to remove the pollutants from the body. A deficiency of dietary vitamins and minerals increased sensitivity to adverse effects of contaminants (Vodela *et al.,* 1998).

Bhatti *et al.* (2016) concluded that, the supplementation of vitamin C in drinking water at the time of vaccination against NDV increase humoral immune response.

These results concluded that dietary supplementation by ascorbic acid or/and dried garlic powder might be useful in reversing the adverse effect on hematological parameters and IgG and IgM induced by cadmium and alleviating the adverse effect of cadmium on immunity.

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الملخص العربى

تأثير التعرض الغذائى للكادميوم لفترة طويلة على بعض الصفات الهيماتولوجية والمناعية فى السمان اليابانى والتاثيرات الوقائية المحتملة لحمض الاسكوربيك والثوم

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أجريت هذه التجربة بإستخدام ٢٠٠ كتكوت ياباني عمر اسبوع غير مجنس وذلك لبيان تأثير تقليل سمية الكادميوم على بعض الصفات الهيماتولوجيه والمناعية بإستخدام حامض الاسكوربيك ومسحوق الثوم المجفف. تم تقسيم السمان إلى خمسه مجموعات تجريبية بكل مجموعة ٤٠ كتكوت تم تقسيمهم في اربعه مكررات بكل مكرره ١٠ كتاكيت في تصميم عشوائي تام . المجموعة الأولى غذيت على عليقة أساسية بدون أي إضافات وإستخدمت كمجموعة كنترول , المجموعة الثانية غذيت على عليقة أساسية مضافا إليها ٤٠ مللجرام كادميوم / كجم علف , المجموعة الثالثة والرابعة والخامسة غذيت على عليقة أساسية مضافا إليها ٤٠ مللجرام كادميوم و٢٠٠مللجرام حامض الاسكوربيك و٥٠٠ مللجرام مسحوق ثوم مجفف و٢٠٠ مللجرام حامض الاسكوربيك + ٥٠٠ مللجرام مسحوق ثوم مجفف على التوالي .وأوضحت النتائج أن الكادميوم خفض معنويا عدد كريات الدم الحمراء وتركيز الهيموجلوبين وحجم كرات الدم الحمراء المضغوط مقارنة بمجموعة الكنترول والتي لم تتغذي على الكادميوم بينما لوحظ زيادة معنوية في عدد كريات الدم البيضاء في حين أن إضافة حامض الاسكوربيك ومسحوق الثوم المجفف إلى الكادميوم حسن معنويا من عدد كريات الدم الحمراء والهيموجلوبين بينما كريات الدم البيضاء و وحجم كرات الدم الحمراء المضغوط لم يتأثر معنويا نتيجة الإضافات السابقة المستخدمة في هذه الدراسة على الجانب الاخر الأمينوجلوبين من النوع G إنخفض معنويا بينما انخفض رقميا الامينوجلوبين من النوع M في المجموعة المغذاة على الكادميوم واوضحت النتائج ان إمداد العليقة المحتوية على الكادميوم بحامض الاسكوربيك ومسحوق الثوم المجفف قد حسن من الصفات السابقة.