MRVSA 5(1), 8-17. Latif and Noordin, 2016
http://mrvsa.com/ ISSN 2307-8073

Mirror of Research in Veterinary Sciences and Animals (MRVSA)

Original article

Influence of dietary garlic supplementation on antioxidant status in broiler chickens exposed to benzo[a]pyrene

I.K. Latif [1*] and M.M. Noordin [2]

[1]Department of Pathology and Poultry Diseases, Faculty of Veterinary Medicine, University of Kerbala, Iraq; [2]Department of Pathology and Microbiology, Faculty of Veterinary Medicine, University Putra Malaysia. *Corresponding author: E-mail address: *lateef.e.k@uokerbala.edu.iq*

Abstract

Garlic is a popular spice added to several edible preparations and is a remedy for a variety of ailments. This experiment was performed to evaluate the effect of fresh garlic meal on the antioxidant status of broiler chickens exposed to an air pollutant benzo[a]pyrene (BaP). Ninety six 1-day-old broiler chicks were divided into 4 equal groups, including control, garlic, BaP, and BaP with garlic. Plasma malondialdehyde (MDA), glutathione (GSH) levels, glutathione peroxidase (GSH-Px), superoxide dismutase (SOD) and catalase (CAT) activities in RBCs hemolysate and liver tissue homogenate were determined. The results of this study revealed a significant alteration in comparison to other groups with respect to MDA, GSH, GSH-Px, SOD and CAT in the group receiving BaP alone demonstrated evidence of oxidative stress, and addition of fresh garlic was significantly reduces these change with potent effects seen after 21 to 35 days in treated group, these functions may be due to increased garlic's antioxidant activities.

Keywords: Garlic; antioxidant status; benzo[a]pyrene; broilers.

To cite this article: I.K. Latif1 and M.M. Noordin (2016). Influence of dietary garlic supplementation on antioxidant status in broiler chickens exposed to benzo[a]pyrene. MRVSA. 5 (1), 8-17.

Introduction

Although garlic (*Allium sativum* L.) has been used for remedies and food for more than a thousand years, it still being utilized and accepted in modern day medicine (Padhye *et al*., 2008), to prevent and treat of a variety of diseases ranging from infections to heart diseases (Thomson and Ali, 2003). The antioxidant properties of garlic are well documented (Pedraza-Chaverri *et al*., 2000). On the other hand, it has been founded that garlic as a natural feed additive, improved broiler's growth, feed conversion ratio, haematilogical performance and decreased mortality rate (Javandel *et al*., 2008; Toghyani *et al*., 2011). It is found that herbal plant has been alleviating BaP-induced immunotoxicity in broilers (Latif *et al*.,

2011). The BaP is a one of potent mutagen, carcinogen, and/or developmental toxicant (Castellano *et al*., 2003). Our findings indicated that intra-tracheal (i.t.) administration of the 15 mg/kg BW of BaP impairs the non-specific respiratory defense mechanism and induce hemato-and hepatotoxicity in broilers (Latif *et al*., 2009; 2010). It also decreased the antioxidant protection as a result of generation reactive oxygen species (ROS) (Briede *et al*., 2004). ROS are continually generated in the body and they are able to damage biological molecules such as DNA, proteins and lipids by triggering chain reactions that the *in vivo* antioxidants work to counterbalance. The oxidative stress is manifested primarily *via* MDA concentration which is the end products formed as a result of lipid peroxidation reactions. This provides direct information on the severity of oxidative stress. In addition, the alteration of antioxidant enzyme activities such as the GSH-Px, SOD and CAT and the reductions of some non-enzymatic antioxidants such as the GSH also provides information on oxidative stress (Evans and Halliwet, 2001; Eraslan, 2007).

One of the potential properties of garlic is the ability of one or more of its constituents to reduce toxicity due to its antioxidant activities (Milner, 2006). There is a lack of information on the effects of garlic in alleviating toxic effects of BaP in non-mammalian species. Thus, the aim of this work was to evaluate the effects of fresh garlic meal to enhance the general antioxidant status against BaP exposure in broilers.

Materials and methods

Experimental animals

Ninety six-day-old, broiler chicks were purchased from a local hatchery. Upon arrival, the chicks were divided randomly into four equal groups of 24 chicks each. The chickens were raised according to routine management practice. All nutrients including water were supplied *ad libitum* to meet the requirements of NRC (1994).

Experimental protocol

Immediately upon arrival, the control groups were given tricaprylin alone for 5 consecutive days intra-tracheal (i.t.) administration by using a micropipette, and fed on normal commercial basal broiler diet only or with additional garlic at the rate of 20 g/kg diet. The BaP groups were instilled with BaP 15 mg/kg BW initially dissolved in tricaprylin as vehicle by the same period, route and fed either a normal commercial basal broiler diet only, or with additional garlic. Before being sacrificed at days 7, 14, 21 and 35 post inoculation (p. i.), blood samples were collected and then 6 birds/groups were killed by cervical dislocation.

Plasma: Blood in EDTA tubes was centrifuged at 3000 rpm for 15 minutes at 4°C. The plasma was separated from the blood and packed in individual Eppendorf tubes, and stored at -20°C until analysis. The plasma was used for the estimation of MDA.

Hemolysate preparation and haemoglobin concentration

Blood samples were centrifuged, then erythrocytes were washed three times with 0.9% normal saline, and 20% (v/v) hemolysate was prepared to measure haemoglobin, GSH level GSH-Px, SOD, and CAT activities.

Liver tissue homogenate preparation and protein concentration

Liver was quickly removed after the chickens were killed. For obtaining tissues supernatants, 1 gram of liver tissue was homogenized in 9 ml of ice-cold KH_2PO_4 containing 1.15% potassium chloride. After centrifugation at 15, 000 rpm for 20 min, supernatant fraction was used to determine the MDA and GSH levels GSH-Px, SOD and CAT activities. The protein concentration of tissue homogenate was determined using bicinchoninic acid protein assay reagents (BCA™ Protein Assay Kit) with reference to bovine serum albumin acted as the standard.
The measurement of MDA concentration in plasma and tissue (liver) were performed by the thiobarbituric acid (TBA) reactive substances assay as described by Ohkawa *et al*. (1979) and its absorbency was measured in a spectrophotometer (Genesys 10 UV Thermo Spectronic Rochester, NY, USA) at 532 nm. The concentration of MDA was expressed as nmol/ml of plasma or nmol/mg protein. The erythrocyte and liver tissue GSH levels were measured using the method described by Beutler *et al*. (1963). The optical density was measured at 412 nm in the spectrophotometer. The erythrocyte and liver tissue GSH-Px activity were measured by the method of Paglia and Valentine (1967). The change in absorbance was recorded at 340 nm at an interval of 30 s for 3 min. The erythrocyte and liver tissue SOD activity were measured according to the method of Marklund and Marklund (1974). The rate of spontaneous oxidation was measured in spectrophotometer at 330 nm. The erythrocyte and liver tissue CAT activity were measured as described by Aebi (1984) and the absorbance was recorded at every 15 s for 1 min at 240 nm against a phosphate buffer blank. All determinations were made in duplicates.
The data were analyzed by using a one way analysis of variance. Differences between means were determined using Tukey's test at $P<0.05$ level.

Results

The levels of MDA in the plasma and liver are shown in Table 1. However, higher ($P < 0.05$) levels of plasma and liver MDA were only seen in the BaP groups throughout the entire experimental period. Nevertheless, the plasma MDA level in the BaP + G group was comparable to that of the control and garlic groups at day 21 and 35. Following this, the highest liver ($P < 0.05$) level of MDA was attained in the BaP until the end of the experimental period.

Table 2 shows the GSH levels of broilers throughout the experimental period. Commencing from day 7 the broilers from the BaP and BaP + garlic groups have the highest ($P<0.05$) GSH levels. On other hand, at days 21, 35 this group has the lowest ($P<0.05$) GSH levels. At these instants, GSH level in the BaP + garlic group was higher ($P<0.05$) than the BaP groups at days 21and 35.

Table 1. The MDA levels plasma and liver tissue homogenate throughout the experimental period (mean ± SD)

		Days p. i.			
Parameters	**Groups**	**7**	**14**	**21**	**35**
MDA plasma (nmol/ml)					
	Control	1.762± 0.260[a]	1.821± 0.100[a]	1.892± 0.138[a]	1.927± 0.138[ab]
	garlic	1.692± 0.141[a]	1.722± 0.073[a]	1.771± 0.144[a]	1.782± 0.144[a]
	BaP	2.953± 0.171[b]	2.975± 0.357[c]	2.604± 0.270[b]	2.252± 0.270[b]
	BaP + garlic	2.725± 0.269[b]	2.521± 0.263[b]	2.049± 0.130[a]	1.892± 0.130[ab]
MDA liver nmol/mg) Protein)	Control	3.992± 0.176[a]	3.940± 0.133[a]	4.038± 0.178[a]	4.021± 0.125[ab]
	garlic	3.917± 0.084[a]	3.734± 0.101[a]	3.880± 0.136[a]	3.752± 0.157[a]
	BaP	5.981± 0.250[b]	6.018± 0.103[c]	5.671± 0.298[c]	4.438± 0.298[c]
	BaP + garlic	5.788± 0.491[b]	5.008± 0.181[b]	5.023± 0.283[b]	4.228± 0.283[bc]

a, b, c Values bearing similar superscript in the same column do not differ at (P<0.05)

Table 2. The glutathione (GSH) levels in RBCs hemolysate and liver tissue homogenate throughout the experimental period (mean ± SD)

		Days p. i.			
Parameters	**Groups**	**7**	**14**	**21**	**35**
GSH µmol/ mg Hb					
	Control	9.28 ± 1.410[b]	12.81 ± 1.234[a]	13.77 ± 1.305[b]	15.38 ± 1.681[ab]
	garlic	9.64 ± 1.259[b]	14.77 ± 1.422[a]	16.84 ± 1.694[a]	18.96 ± 2.102[a]
	BaP	15.7± 2.132[a]	11.97 ± 1.649[a]	8.225± 2.006[c]	10.94 ± 1.710[c]
	BaP + garlic	14.5± 1.888[a]	12.59 ± 1.841[a]	14.13 ± 1.503[b]	14.02 ± 1.441[b]
GSH mmol/ mg protein	Control	12.4± 1.182[b]	14.42 ± 1.342[a]	16.82 ± 1.647[b]	17.00± 2.092[ab]
	garlic	12.7± 1.142[b]	15.91 ± 1.448[a]	18.11 ± 2.112[a]	18.98± 1.929[a]
	BaP	18.0± 1.769[a]	13.65 ± 1.662[a]	11.65 ± 1.074[c]	12.10 ± 1.420[c]
	BaP + garlic	16.3± 1.522[a]	13.52 ± 1.558[a]	14.68± 1.540[b]	15.37± 1.551[b]

[a, b, c] Values bearing similar superscript between column do not differ at (P<0.05)

The control and garlic groups exhibited an increment of GSH-Px as time advanced, fluctuations were seen in the BaP and BaP + garlic groups (Table 3). However, at day 7 the BaP and BaP + garlic groups were significantly ($P<0.05$) increased than any other groups. After this, from days 21, 35 the BaP group has the lowest ($P<0.05$) GSH-Px level than garlic group, such difference was comparable to the control at day 14. At these instants, GSH-Px level in the BaP + garlic group was significantly ($P<0.05$) different from BaP group at days 21 and 35 and such difference was comparable in the GSH-Px of RBCs hemolysate.

The SOD activities of broilers during the experimental period are shown in Table 4. Despite the broilers from the BaP and BaP + garlic groups have the highest ($P<0.05$) activities from all other groups day 7. Such differences were comparable to all groups on day 14. The BaP + garlic group has the highest ($P<0.05$) activity of SOD in RBCs haemolysate than BaP group at day 21, such differences were comparable to all groups on day 35.

Table 3. The glutathione peroxidase (GSH-Px) activities in RBCs hemolysate and liver tissue homogenate throughout the experimental period (mean ± SD).

		Days p. i			
Parameters	**Groups**	**7**	**14**	**21**	**35**
GSH-Px U/g Hb					
	Control	1.959 ± 0.335^{b}	2.198 ± 0.394^{a}	2.685 ± 0.513^{ab}	2.742 ± 0.632^{b}
	garlic	2.133 ± 0.463^{b}	2.369 ± 0.420^{a}	3.462 ± 0.683^{a}	4.062 ± 0.615^{a}
	BaP	3.602 ± 0.618^{a}	3.218 ± 0.652^{a}	1.886 ± 0.338^{b}	2.428 ± 0.469^{b}
	BaP+garlic	3.461 ± 0.505^{a}	3.091 ± 0.505^{a}	2.546 ± 0.405^{ab}	3.365 ± 0.587^{ab}
GSH-Px U/g protein	Control	4.980 ± 0.690^{b}	6.063 ± 0.570^{b}	8.006 ± 0.600^{b}	9.25 ± 0.877^{b}
	garlic	5.903 ± 0.824^{b}	7.543 ± 0.620^{a}	10.78 ± 0.849^{a}	12.15 ± 1.216^{a}
	BaP	8.931 ± 0.793a	9.088 ± 1.210^{a}	6.208 ± 0.524^{c}	7.389 ± 0.735^{c}
	BaP +garlic	8.609 ± 0.843^{a}	8.279 ± 0.949^{a}	8.304 ± 0.847^{ab}	9.417 ± 0.940^{b}

$^{a, b, c}$ Values bearing similar superscript between column do not differ at ($P<0.05$).

Table 4. The superoxide dismutase (SOD) activities in RBCs hemolysate and liver tissue homogenate throughout the experimental period (mean ± SD).

		Days p. i.			
Parameters	**Groups**	**7**	**14**	**21**	**35**
SOD U/g Hb					
	Control	17.17 ± 2.589^{b}	19.72 ± 2.937^{a}	20.59 ± 2.796^{a}	22.42 ± 3.548^{ab}
	garlic	17.03 ± 3.164^{b}	21.28 ± 2.830^{a}	27.38 ± 3.334^{a}	30.68 ± 5.101^{a}
	BaP	27.15 ± 5.699^{a}	25.66 ± 5.507^{a}	15.72 ± 1.849^{b}	19.39 ± 2.793^{b}
	BaP+ garlic	28.69 ± 5.548^{a}	21.90 ± 4.393^{a}	20.80 ± 3.589^{ab}	25.11 ± 4.239^{ab}
SOD U/g protein	Control	41.33 ± 6.700^{b}	46.85 ± 6.988^{a}	50.35 ± 7.689^{a}	55.44 ± 8.600^{ab}
	garlic	43.92 ± 7.390^{b}	48.55 ± 8.571^{a}	58.06 ± 9.506^{a}	60.38 ± 9.550^{a}
	BaP	72.44 ± 10.61^{a}	59.39 ± 9.792^{a}	32.77 ±7.088^{b}	41.08 ± 7.299^{b}
	BaP + garlic	69.60 ± 9.689^{a}	57.28 ± 8.155^{a}	47.38 ± 8.948^{ab}	56.83 ± 8.110^{ab}

$^{a, b}$ Values bearing similar superscript between column do not differ at (P<0.05).

Table 5 shows the CAT activities of broilers during the experimental period. However, higher (P<0.05) levels of CAT were seen in the BaP and BaP + garlic groups at day 7. Nevertheless, the CAT level in the BaP group was the lowest (P<0.05) from day 21 to the end of the experimental period. At day 14, the CAT activities remained comparable between all groups. At these instants, CAT activities in the BaP + garlic group was significantly (P<0.05) different from BaP group at days 21and 35.

Table 5. The catalase (CAT) activities in RBCs hemolysate and liver tissue homogenate throughout the experimental period (mean ± SD)

		Days p. i.			
Parameters	**Group**	**7**	**14**	**21**	**35**
CAT U/ g Hb					
	Control	18.66 ± 2.340^{b}	18.74 ± 2.697^{a}	23.77 ± 3.094^{b}	27.17 ± 4.370^{a}
	garlic	19.83 ± 2.479^{b}	24.28 ± 3.876^{a}	30.28 ± 5.450^{a}	29.55 ± 5.153^{a}
	BaP	29.40 ± 4.209^{a}	27.90 ± 4.109^{a}	14.12 ± 2.994^{c}	16.72 ± 4.111^{b}

	BaP+ garlic	29.98 ± 3.520[a]	24.44 ± 3.282[a]	22.11 ± 4.101[b]	24.42 ± 5.112[a b]
CAT U/g protein	Control	42.65 ± 6.805[b]	50.75 ± 7.135[b]	57.70 ± 7.380[ab]	58.60 ± 7.776[b]
	garlic	40.48 ± 6.399[b]	54.49 ± 6.774[ab]	70.79 ± 9.434[a]	77.72 ± 9.557[a]
	BaP	72.11± 9.680[a]	68.82 ± 8.684[a]	37.43 ± 6.672[c]	40.56 ± 7.182[c]
	BaP + garlic	75.79 ± 9.911[a]	65.20 ± 9.070[ab]	54.28 ± 6.221[b]	56.95 ± 7.828[b]

[a, b, c] Values bearing similar superscript between column do not differ at (P<0.05).

Discussion

Under usual conditions, the production of reactive free radicals and their elimination are in a dynamic equilibrium. This balance could be disturbed when the generation of free radicals becomes higher than the protection capacity of systemic antioxidant defence. The impaired equilibrium in favor of oxidants is named oxidative stress and it is involved in the pathogenesis of numerous diseases, this in turn probably incited increased oxidative stress, as substantiated by increased MDA levels (Halliwell and Gutteridge, 2007).

Our experiment indicate that BaP exposure induced significant alteration in antioxidant defense of the broiler chickens, determined by observable increased in the plasma and liver MDA levels during the experimental period (Table 1) and endogenous level of GSH and the activities of GSH-Px, SOD and CAT enzymes at day 7 p.i. in comparable with the control (Table 2-5). As time advanced (days 21 and 35) the body cannot able to manufacture these antioxidant with enough quantity to reduce the oxidative stress result by BaP (Table 2-5), reflects an imbalance between the production and scavenging of those oxidants. GSH is the major non-enzymatic antioxidant in cells and can effectively scavenge free radicals either directly or indirectly through enzymatic reactions. It is plays an important role in antioxidant defence, nutrient metabolism and regulation of cellular events. In addition to GSH, antioxidant enzymes GSH-Px, SOD, and CAT can also play an important part in coping with oxidative stress (Valavanidis *et al*., 2006).

Much attention has been given to herbal medicine (Rajesh *et al*., 2012), our result showed that garlic at 20 g/kg diet enhanced the activities of GSH-Px, SOD and CAT ($P < 0.05$) on the RBCs haemolysate and liver tissue homogenate was detected in the treated group after 21 days (Table 2-5) in compared to the BaP group. These results suggest that the enhancement of antioxidant enzymes can also contribute to protection against BaP exposure (Amagase *et al*., 2009).

A key mechanism for the multiple effects of ROS is the activation of redox-regulated gene regulatory proteins (Lavrovsky *et al*., 2000) that turn on genes for

pro-inflammatory enzymes such as cyclooxygenase (COX) and lipoxygenase (LOX) (Bozin *et al*., 2008). Redox-regulated genes are controlled by reduction (via antioxidants) and oxidation (via ROS) of components of the signal transduction pathways that control their expression. Expression of COX is up regulated by a surplus of ROS and down regulated by antioxidants such as those present in the garlic.

This particularly study indicated that inclusion of 20 g/kg garlic, with its antioxidant characteristics, might be developed as an effective preventive and therapeutic agent able to slow down, stop, or reverse oxidation processes by scavenging oxidizing agents of BaP. In conclusion, garlic may have protective effects against BaP induced oxidative stress, which can be mainly attributed to restoration of the GSH level. Enhancement of the activities of GSH-Px, SOD and CAT may also contribute to these protective effects; further investigations in different situations should be conducted to achieve more comprehensive results.

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