Efficacy of *Negilla sativa* meal in modulating the oxidative stress induced by benzo[a]pyrene exposures in broilers

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Abstract:

Nigella sativa (Ns) has been employed for thousands of years as a spice and food preservative and commonly used in folk medicine all over the world for the treatment and prevention of a number of diseases and conditions. The aim of the study was as an attempt to investigate the effect of Ns meal on the oxidative stress (OS) following intra-tracheal (IT) administration of an air pollutant benzo[a]pyrene (BaP) in broiler chickens. At day one of age, chicks were divided into four groups comprising of 24 birds each, as controls, Ns, BaP-only, and BaP with Ns. The antioxidant function of glutathione (GSH), glutathione peroxidase (GSH-Px), superoxide dismutase (SOD) and catalase (CAT) in RBCs hemolysate and liver tissue homogenate in the group receiving BaP alone demonstrated evidence of OS, and oral supplementation of Ns was significantly reduces these alterations with potent effects seen after 21 to 35 days in treated group which is a key finding from this study. It is concluded that exposure to BaP may exert adverse effects on the erythrocyte and liver antioxidant enzymes of broilers which may increase their susceptibility to disease infection, with a disastrous outcome and oral supplementation of Ns was significantly reduces these effects.

Keywords: *Nigella sativa*; benzo[a]pyrene; oxidative stress; broilers.

فاعلية استخدام الحبة السوداء مع العليقة للتخفيف من حالة الأكسدة الناتجة عن تعرض دجاج اللاحم (benzo[a]pyrene) للبنزو الفا بايرين

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الخلاصة:

استخدمت الحبة السوداء منذ آلاف السنين كبهارارات أو كماده غذائية حافظه، وعادتا ما أستعملت في مناطق واسعة من العالم للوقاية أو العلاج لمجموعه من الأمراض والحالات. كان الغرض من هذه الدراسة هو لمحاولة معرفة بيان تأثير إضافة الحبة السوداء إلى العليقة على حالة الأكسدة الناتجة عن تجريع دجاج اللاحم عن طريق القصبة بالملوث البيئي البنزو الفا بايرين. في عمر يوم واحد قسمت الأفراخ إلى أربعة مجاميع متساوية ، كل مجموعه تحتوي على 24 فرخا، ضمت مجموعة السيطرة ومجموعة الحبة السوداء ومجموعه البنزو الفا بايرين ومجموعه البنزو الفا بايرين مع الحبة السوداء.

حيث أظهرت النتائج إن للبنزو الفا بايرين دور مهم في زيادة الأكسدة من خلال التأثير السلبي على فعالية الإنزيمات المضادة للأكسدة لكريات الدم ونسيج الكبد، كما وان إعطاء الحبة السوداء مع العليقة عن طريق الفم تأثير ايجابي في تقليل هذه الأكسدة من خلال تنظيم فعاليه الإنزيمات المضادة للأكسدة بعد 21 يوما من الإعطاء. نستنتج من هذه الدراسة أن للبنزو الفا بايرين تأثير سلبي على الإنزيمات المضادة للأكسدة لكريات الدم الحمراء والكبد وهذا التأثير قد يودي إلى زيادة الإصابة بالأمراض في دجاج اللاحم وان للحبة السوداء دور مهم في تخفيف هذا التأثير.

Introduction:

Many natural substances have been used as remedies by humans for a long time; such remedies are still used in modern day medicine (1). Herbal medicines have rapidly developed and gained wide acceptance. Most of such herbal drugs have been recorded to have some antioxidative activity (2). Among these natural substances Ns which have a multipurpose medicinal plant used in folk medicine all over the world for the treatment and prevention of a wide number of diseases (3). The potential properties of Ns compound to alleviate the harmful effects are due to their antioxidant activities (4). Feeding growing chicks on diet containing natural feed additives such as Ns have been reported to improved chicks performance, digestibility and decreased abdominal fat (5). The OS is manifested primarily *via* alterations of antioxidant enzyme activities such as the GSH-Px, SOD and CAT and the reductions of some non-enzymatic antioxidants such as the GSH (6). Suppression of the antioxidant defense by PAH, especially BaP through aryl hydrocarbon receptor leads to the generation of reactive oxygen species (7). Our findings indicated that IT administration of the 15 mg/kg BW impairs the non-specific respiratory defense mechanism and induce hemato-and hepatotoxicity in broilers (8, 9). A dearth of knowledge still exists on the OS effect of BaP and the role of Ns meal to enhancing the antioxidant activities in commercial poultry with regards to GSH, GSH-Px, SOD and CAT in RBCs hemolysate and liver tissue changes following IT administration of BaP.

Materials And Methods:

Animals and Experimental Design:

Ninety six-day-old, broiler chicks obtained from a local hatchery were kept at the poultry house, Faculty of Veterinary Medicine, University of Baghdad, from January 2011 till March 2011. Upon arrival, the chicks were divided randomly into four equal groups of 24 chicks each. Then, the control groups were given tricaprylin alone for 5 consecutive days IT by using a micropipette, and fed on normal commercial basal broiler diet only or with additional Ns. 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 Ns. Before being sacrificed at days 7, 14, 21 and 35 PI, blood samples were collected and then 6 birds/groups were killed by cervical dislocation. The chickens were raised according to routine management practice. All nutrients including water were supplied *ad libitum* to meet the requirements of NRC (1994).

Hemolysate Preparation

Blood samples were centrifuged (3000 rpm, 10 min) and the buffy coat and plasma were separated. Erythrocytes were washed three times with 0.9% normal saline and then 20% (v/v) hemolysate was prepared to measure haemolysate haemoglobin and GSH level GSH-Px, SOD, and CAT activities.

Liver tissue homogenate preparation

Liver was quickly removed after the chickens were killed. For obtaining tissues supernatants, 1 gr of liver 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 GSH level GSH-Px, SOD and CAT activities.

Measurement of GSH Levels

The erythrocyte and liver tissue GSH levels were measured using the method described by Beutler et al. (10). Briefly, 0.2 ml sample was added to 1.8 ml distilled water (DW). Three ml of the precipitating solution (1.67 g metaphosphoric acid, 0.2 g EDTA and 30 g NaCl in 100 ml DW) was mixed with sample. The mixture was allowed to stand for 5 min and then filtered. Two ml of filtrate was taken and added into another tube, then 8 ml of the phosphate solution and 1 ml 5,5'-dithiobis 2- nitrobenzoic acid (DTNB) were added. A blank was prepared with 8 ml of phosphate solution, 2 ml diluted precipitating solution, and 1 ml DTNB reagent. A standard solution of the glutathione was prepared (40 mg/100 ml). The optical density was measured at 412 nm in the spectrophotometer.

Measurement of GSH-Px Activity

The erythrocyte and liver tissue GSH-Px activity was measured by the method of Paglia and Valentine (11). The reaction mixture contained 2.49 ml phosphate buffer, 0.1 ml NADPH, 0.1 ml GSH, 0.01 ml sodium azide, 4.6 U glutathione reductase and 10 µl sample. The reaction was initiated by adding 0.1 ml of H_2O_2. The change in absorbance was recorded at 340 nm at an interval of 30 s for 3 min.

Measurement of SOD Activity

The erythrocyte and liver tissue SOD activity was measured according to the method of Marklund and Marklund (12) based on the ability of SOD to inhibit the autoxidation of pyrogallol. Firstly, 0.5 ml of the sample was added to 1.5 ml of ice cold DW followed by

0.5 ml ethanol and then 0.3 ml chloroform, mixed well and centrifuged for 10 min at 3000 rpm. Secondly, following an addition of 2 ml of DW, a mixture of reactive solution was prepared by adding 75 µl chloroform-ethanol extract to 2.25 ml Tris-HCl. The mixture was then kept and 0.15 ml of pyrogallol solution was added. The rate of spontaneous oxidation was measured in spectrophotometer at 330 nm.

Measurement of CAT Activity

The erythrocyte and liver tissue CAT activity was measured as described by Aebi (13). Briefly, 1.98 ml of phosphate buffer and 20 µl of sample was mixed in a cuvette. Reaction was started by adding 1 ml H_2O_2 (30 mM) and the absorbance was recorded at every 15 s for 1 min at 240 nm against a phosphate buffer blank. 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 GSH levels are shown in Table 1. A trend of an increased of GSH levels were seen in the control and Ns groups. Nevertheless, manifested fluctuations in the BaP and BaP + Ns groups during the course of the experiment. Commencing from day 7 the broilers from the BaP and BaP + Ns groups have the highest (P<0.05) GSH levels. At days 21, 35 the BaP group has the lowest (P<0.05) GSH levels. At these instants, GSH level in the BaP + Ns group was significantly (P<0.05) different from BaP groups at days 21, 35.

Table 1. The glutathione (GSH) levels in RBCs haemolysate and liver tissue homogenate during the trial (mean ± SD).

		Days p. i.			
Parameters	Groups	7	14	21	35
GSH µmol/mg Hb	Control	8.82 ± 1.033^{b}	11.31 ± 0.905^{a}	13.21 ± 1.373^{b}	14.92 ± 1.284^{b}
	Ns	8.64 ± 1.150^{b}	13.02 ± 1.223^{a}	17.07 ± 1.224^{a}	19.02 ± 1.409^{a}
	BaP	14.90 ± 1.086^{a}	11.97 ± 1.144^{a}	9.120 ± 0.921^{c}	11.62 ± 0.940^{c}
	BaP + Ns	13.64 ± 1.288^{a}	12.29 ± 1.141^{a}	13.89 ± 1.108^{b}	14.86 ± 1.502^{b}
GSH mmol/mg protein	Control	11.30 ± 1.209^{b}	12.79 ± 1.542^{a}	14.86 ± 1.117^{b}	16.00 ±1.427^{b}
	Ns	11.93 ± 0.943^{b}	14.67 ± 1.448^{a}	17.67 ± 1.328^{a}	18.98 ±1.379^{a}
	BaP	17.68 ± 1.366^{a}	12.34 ± 1.139^{a}	11.20 ± 0.974^{c}	12.87 ± 1.960^{b}
	BaP + Ns	15.36 ± 1.208^{a}	13.07 ± 1.018^{a}	14.68 ±1.241^{b}	15.17 ±1.413^{b}

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

Although the control and Ns groups exhibited an increment of GSH-Px as time advanced, fluctuations were seen in the BaP and BaP + Ns groups (Table 2). However, at day 7 and 14 the BaP and BaP + Ns groups were significantly (P<0.05) increased than

any other group. After this, from days 21, 35 the of BaP group has the lowest GSH-Px (P<0.05) level than control and Ns groups. At these instants, GSH-Px level in the BaP + Ns group was significantly (P<0.05) different from BaP group at days 21, 35 and such difference was comparable in the GSH-Px of RBCs haemolysate to the BaP at day 35.

Table 2. The glutathione peroxidase (GSH-Px) activities in RBCs haemolysate and liver tissue homogenate during the trial (mean ± SD).

		Days p. i			
Parameters	Groups	7	14	21	35
GSH-Px U/g Hb	Control	1.780 ± 0.401^{b}	1.987 ± 0.469^{b}	2.699 ± 0.491^{a}	2.690 ± 0.610^{b}
	Ns	2.003 ± 0.360^{b}	2.197 ± 0.400^{b}	3.590 ± 0.552^{a}	4.143 ± 0.602^{a}
	BaP	3.132 ± 0.519^{a}	3.000 ± 0.312^{a}	1.793 ± 0.391^{b}	2.597 ± 0.391^{b}
	BaP+Ns	2.931 ± 0.463^{a}	3.111 ± 0.463^{a}	2.711 ± 0.400^{a}	3.244 ± 0.611^{ab}
GSH-Px U/g protein	Control	5.881 ± 0.722^{b}	6.651 ± 0.470^{b}	7.462 ± 0.600^{b}	8.954 ± 0.877^{b}
	Ns	5.986 ± 0.814^{b}	7.442 ± 0.720^{ab}	9.983 ± 0.792^{a}	11.60 ± 0.913^{a}
	BaP	9.188 ± 0.930^{a}	8.985 ± 0.974^{a}	5.708 ± 0.722^{c}	6.781 ± 0.555^{c}
	BaP +Ns	8.794 ± 0.793^{a}	9.001 ± 0891^{a}	8.209 ± 0.677^{b}	9.214 ± 0.881^{b}

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

Similarly, an increasing pattern of SOD activities were seen in control and Ns groups as time advanced, fluctuations were seen in the BaP and BaP + Ns groups (Table 3). Commencing from day 7 the broilers from the BaP and BaP + Ns groups have the highest (P<0.05) levels from all other groups. Such differences were comparable to all groups on day 14. The BaP + Ns groups have the highest (P<0.05) levels of SOD activity in RBCs haemolysate from BaP group at day 21 and such differences were comparable to all groups on day 35.

Table 3. The superoxide dismutase (SOD) activities in RBCs haemolysate and liver tissue homogenate during the trial (mean ± SD).

		Days p. i.			
Parameters	Groups	7	14	21	35
SOD U/g Hb	Control	15.20 ± 2.383^{b}	17.15 ± 1.843^{a}	18.80 ± 2.109^{b}	20.07 ± 2.394^{b}
	Ns	15.78 ± 1.869^{b}	20.63 ± 2.699^{a}	25.18 ± 3.341^{a}	28.06 ± 5.047^{a}
	BaP	22.63 ± 2.699^{a}	19.68 ± 2.840^{a}	14.22 ± 1.903^{c}	18.98 ± 2.903^{b}
	BaP+Ns	19.94 ± 2.530^{a}	18.86 ± 3.102^{a}	19.17 ± 2.250^{b}	23.03 ± 3.49^{ab}
SOD U/g protein	Control	39.33 ± 4.986^{b}	43.93 ± 6.473^{a}	47.35 ± 7.871^{ab}	51.44 ± 8.690^{ab}
	Ns	39.92 ± 5.100^{b}	49.01 ± 8.091^{a}	56.06 ± 9.030^{a}	58.38 ± 9.675^{a}
	BaP	70.44 ± 8.671^{a}	58.44 ± 9.308^{a}	35.77 ± 8.875^{b}	40.08 ± 7.186^{b}
	BaP +Ns	66.60 ± 9.209^{a}	56.38 ± 7.168^{a}	50.38 ± 7.168^{ab}	51.83 ± 8.219^{ab}

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

The data of CAT activities in RBC haemolysate and liver tissue homogenate during the experimental period are presented in Table 4. However, higher (P<0.05) levels of CAT were seen in the BaP and BaP + Ns 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 GSH level remained comparable between all groups. At these instants, CAT level in the BaP + Ns group was significantly (P<0.05) different from BaP group at days 21, 35.

Table 4. The catalase (CAT) activities in RBCs haemolysate and liver tissue homogenate during the trial (mean ± SD).

		Days p. i.			
Parameters	Groups	7	14	21	35
CAT U/g Hb	Control	14.49 ± 2.141^{b}	17.25 ± 2.391^{a}	21.58 ± 3.094^{b}	27.10 ± 4.370^{a}
	Ns	16.15 ± 2.108^{b}	22.41 ± 3.270^{a}	29.13 ± 4.460^{a}	29.67 ± 4.153^{a}
	BaP	22.93 ± 3.307^{a}	20.83 ± 3.095^{a}	14.63 ± 2.394^{c}	15.39 ± 2.791^{b}
	BaP+Ns	21.90 ± 3.000^{a}	21.25 ± 2.881^{a}	20.61 ± 2.181^{b}	24.52 ± 3.852^{a}
CAT U/g protein	Control	47.53 ± 7.192^{b}	51.63 ± 5.130^{a}	63.00 ± 7.389^{ab}	62.00 ± 7.306^{b}
	Ns	46.19 ± 6.119^{b}	59.89 ± 5.679^{a}	76.84 ± 8.130^{a}	81.10 ± 9.977^{a}
	BaP	77.83 ± 9.005^{a}	65.44 ± 8.147^{a}	40.31 ± 7.164^{c}	45.31 ± 7.164^{c}
	BaP +Ns	80.00 ± 8.937^{a}	69.44 ± 9.110^{a}	59.63 ± 6.630^{b}	60.69 ± 6.864^{b}

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

Discussion:

The OS constitutes an important mechanism of biological damage in live animals and it is regarded as the cause of several pathologies that affect poultry growth (6). It is proved that BaP decreased the antioxidant protection, due to generation reactive oxygen species result of OS (7). Our result showed that BaP exposure induced significant alteration in antioxidant defence of the broilers, determined by observable increased in the level of GSH and the activities of GSH-px, SOD and CAT at day 7 PI (Table 1- 4). As time advanced (days 21 and 35) the body cannot able to produce these antioxidant with enough quantity to reduce the OS result by BaP (Table 1- 4), reflects an imbalance between the production of oxidants and scavenging or removal of those oxidants. The decrease of the activities of antioxidant enzymes could have a negative impact on cellular resistance against the oxidant induced damage of cell genome and cell killing (6). The GSH and the activities of GSH-px, SOD are involved in the antioxidant defence system in a first attempt to control and eliminate the toxic ROS. On the other hand, Popova and

Popov (14) reported that the antioxidant enzyme catalase was important for adaptation of cells to OS and preserved cells *via* degradation of the reactive hydrogen peroxide.

Ns is a multipurpose medicinal plant used in folk medicine for the treatment and prevention of a wide number of diseases (3). One of the potential properties of Ns seeds is to reduce OS due to its antioxidant activities **(**4).

As seen in the present study, evidence for a reduced the effect of BaP on the RBCs haemolysate and liver tissue homogenate was detected in the Ns treated group, due to partially improvement of the broilers antioxidant defence (days 21 and 35) by modulating OS and improved the antioxidant systems (Table 1- 4) in compared to the BaP group. It is likely that the antioxidant properties of Ns seed protected the haematopoietic tissues against OS, and ameliorated the reduction of antioxidant capacity leading to better antioxidant scavenging systems. These results are consistent with those of Sogut et al. (15) who reported that Ns in broiler chickens might be used as a potent antioxidant to prevent liver from oxidative stress resulting lipidperoxidation. On the other hand, Ns seed extract protects erythrocytes against lipid peroxidation, protein degradation, loss of deformability, and increased osmotic fragility caused by H_2O_2 (16) and this could be attributed to its antioxidant properties.

In summary, the present study provides evidence that exposure to BaP induced OS in broilers *via* impair the antioxidant activities and supplementation of Ns meal was efficacious *via* modulating these effects due to their antioxidant properties.

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