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Efficacy of *Nigella sativa* in alleviating benzo[a]pyrene-induced immunotoxicity in broilers

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Summary. The immune response of broiler chickens exposed to intra-tracheal (i.t.) administration of benzo[a]pyrene (BaP) with and without *Nigella sativa* (Ns) supplementation was investigated. A total of 120 day-old chicks were divided into four groups comprising 30 birds each, into a control, Ns, BaP, and BaP+Ns group. Immune responses to Newcastle disease (ND) were evaluated by haemagglutination inhibition (HI), phytohaemagglutinin (PHA) skin test and carbon clearance assay (CCA). In most instances, there was a significant increase ($p<0.05$) in the ND-HI antibody titers, PHA skin-swelling response and phagocytic activity in the BaP + Ns group compared to that of the BaP group. Likewise, organ weight and indices of the spleen, bursa of Fabricius and thymus of birds from the BaP + Ns group were also higher ($p<0.05$) than that of the BaP group from day 1 until day 21. It is concluded that exposure to BaP may exert adverse effects on the immune system of broilers which may increase their susceptibility to disease, and Ns supplementation significantly reduces these alterations.

Key words: *Nigella sativa*, Benzo[a]pyrene, Immunity, Broilers

Introduction

Many anciently claimed natural remedies are still being ultilised and accepted in modern day medicine (Padhye et al., 2008). Whole fruits and vegetables give benefits that are generally greater than their individual components (Liu and Sun, 2003). These so-called herbal remedies have either a direct or indirect effect on immunomodulation (Villasenor-Garcia et al., 2004; Lin et al., 2006) and directly stimulate the proliferation of B lymphocytes (Choi et al., 2004) as well as some level of anti-oxidative activity (Amagase et al., 2009). Among these natural substances, Ns is an annual herbaceous plant of the *Ranunculaceae* family which have been used traditionally for centuries in many parts of the world for treating various diseases (Phillips, 1992). Pharmacological investigations revealed that Ns seed has a wide spectrums of properties, among others anti-inflammatory, antidiabetic, analgesic and antibacterial (Khalife and Lupidi 2007; Ramadan, 2007). Among the active constituents found in Ns are fixed and essential oils, proteins, alkaloids and saponin including nigellone (El_Dakhakhnya et al., 2002) and thymoquinone (Ali and Blunden, 2003). Sogut et al. (2008) have shown that Ns in might be used as a potent antioxidant and enhance the immune system broiler chickens. Importantly, the seeds have been reported to be safe when used orally in moderate amount (DerMarderosian et al., 2005).

The sensitivity of the immune system to environmental contaminants (Grasman, 2002) may lead to functional modifications (Zhang et al., 2009). The BaP is a ubiquitous immunotoxic environmental contaminant (Carlson et al., 2004) that is often used as a model in polycyclic aromatic hydrocarbon (PAH) toxicity studies (Boström et al., 2002; De Buck et al., 2005).

Cytochrome P4501A substrate of BaP is also believed to act as an immunosuppressant in various species (Gupta and Abou-Donia, 1998). Recently BaP have been documented to impair the non-specific respiratory defense mechanism and induce hemato- and hepatotoxicity in broilers (Latif et al., 2009, 2010).

There issue a lack of knowledge still exists on the mechanism of immunosuppression of BaP and the role of Ns in immunomodulation in commercial poultry, since chickens form a good model in elucidating toxic mechanism of environmental pollutants (Gupta and Abou-Donia, 1998). This research was undertaken to evaluate the effect of Ns supplementation on the immune

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response in broilers with regards to humoral and cell-mediated immunity, macrophage activity and selected haemogram changes following i.t. administration of BaP.

Materials and methods

Experimental animals

The experimental procedures were approved by the Faculty of Animal Care and Use committee (Approval No.: 08R29/July08-Jun09). One hundred and twenty newly hatched (Cobb strain) chicks obtained from a local hatchery were kept at the Department of Animal Science, Universiti Putra Malaysia. Upon arrival, the chicks were weighed and divided randomly into four equal groups of 30 chicks each in cages of three tiered batteries with wire floors. The chickens were raised according to routine practice in terms of light and temperature. Water and feed were given ad libitum and nutrients supplied in accordance with the NRC requirements (1994).

Experimental protocol

Immediately upon arrival, six chicks from each group were bled via cardiac puncture and serum samples were determined for maternally-derived antibody titers against ND. All chickens were vaccinated with commercial live ND vaccine (LaSota strain) via intraocular route on day 7 and this was repeated on day 21. The control groups were given tricaprylin (ICN, Costa Mesa, CA, USA) alone for 5 consecutive days i.t. using a micropipette (Eppendorf, Germany) and fed on normal commercial basal broiler diet alone or with additional Ns. The remaining groups were administered with BaP (Sigma, St. Louis, MO, USA, 15 mg/kg BW initially dissolved in tricaprylin) at the same duration, route and fed either a normal commercial basal broiler diet alone or supplemented with Ns. In all treatments, the total volume of inoculums administered to each chick was 100 μl. Before being sacrificed at days 14, 21 and 35 p.i., blood samples were collected via the heart and then 8 birds per group were killed by cervical dislocation.

Body weight, feed intake and feed conversion ratio

Weekly individual body weight (BWG), feed intake (FI) and feed conversion ratio (FCR) were recorded and calculated, respectively, throughout the experiment.

Haemogram and serum biochemistry

Blood samples were collected via cardiac puncture into EDTA and plain vacutainer tubes. The EDTA blood samples were used for total and differential white blood cell (WBC) counts in a blood counter (CELL-DYN® 3700, Abbott Diagnostics, IL, USA) using its standard reagent (Abbott Diagnostics, USA). Sera obtained from the plain tubes were used in the determination of TP and A/G ratio in an automated analyzer (Hitachi 902, Japan), and ND-HI titer.

Haemagglutination Inhibition (HI) titer

The ND-HI titer was done according to the method described by Allan and Gough (1974).

Phytohaemagglutinin assay (PHA)

The PHA skin test was done at days 14, 21 and 35 p.i. by injecting 0.2 mg PHA-P (Sigma L-8754, St. Louis, MO USA) into the patagium and measurement of the subsequent swelling was used as an assay of *in vivo* T-cell mediated immune responsiveness (Cheng and Lamont, 1988). Following the recommendations of Martin et al. (2006), we did not inject PBS to measure the swelling as a control in the other wing. Instead, the thickness of the right wing-web to the nearest 0.01 mm with a micrometer (TESA Shop Cal, TESA Technology, Switzerland) was measured immediately before and 24 h after injection (Smits et al., 1999).

Carbon clearance assay (CCA)

The CCA was also done at days 14, 21 and 35 p.i. as described by Heller et al. (1992) using India ink (Pelikan A.G., Hanover, Germany). Briefly, a 100 μl blood sample was collected from the opposite wing vein, before and 3, 7 and 15 min post carbon solution injection. The absorbance of the supernatant was measured at 675 nm using a spectrophotometer (Genesys 10 UV Thermo Spectronic Rochester, NY, USA) and values measured according to this formula:
OD % = [(OD reading at a considered time - OD reading at 0 min)/OD reading at 0 min] x 100.

Lymphoid organs weight and indices

Eight birds from each group were sacrificed via cervical dislocation at days 14, 21, and 35 p.i. Following a thorough visual appraisal, the spleen, bursa and thymus were immediately removed, stripped of fat and connective tissue, blotted dry and individually weighed. Since substantial lymphoid organ weight change was anticipated, their indices were calculated (Sellers et al., 2007).

Histopathology

Spleen, bursa and thymus were fixed overnight in 10% buffered formalin, dehydrated in ascending grades of alcohol and processed in the routine manner, sectioned at 4 μm, and stained with Mayer's Haematoxylin and Eosin (H&E) in accordance with the guidelines for the standard procedure and evaluated by

light microscopy.

Statistical analysis

The results were presented as mean ± standard deviation (SD) and data were subjected to ANOVA. Differences between means were determined using Tukey's test in which the significance level was designated at $p<0.05$.

Results

All groups registered an increase in BW (Table 1) throughout the experiment. Commencing from day 7 until the end of the experiment, the BW of broiler from the BaP group was almost always the lowest ($p<0.05$). That of the BaP + Ns was higher ($p<0.05$) than the BaP at day 21 only.

Table 2 shows the BWG of birds throughout the experimental period. The lowest ($p<0.05$) BWG was seen in the BaP at the period of 1-21 and 1-35 days. However, the gain remained comparable to that of the BaP + Ns at the period of 1-21 and 1-35 days, respectively.

Table 3 shows the FI and FCR of broilers during the experimental period. Broilers from the BaP group have the significantly ($p<0.05$) differing FI and FCR at all times compared to the control. It was comparable to the Ns group at day 1-35 FCR and its FI and FCR being similar to the BaP + Ns group at days 21-35 and 1-35. However, the BaP + Ns group differs ($p<0.05$) from the control in FI (days 1-35) and FCR (days 21-35; 1-35). Last but not least, the BaP + Ns group has higher ($p<0.05$) FCR than that of the Ns group at day 1-35.

The total WBC counts and H/L ratio of broilers during the experimental period is shown in Table 4. Despite being comparable to the BaP + Ns group, the total WBC count of the broilers from the BaP group was always the lowest ($p<0.05$) compared to the control and Ns group from day 7 until day 21. However, the WBC count of the BaP + Ns group was higher ($p<0.05$) than the control group at days 7 and against the Ns group at days 7 and 14. Similarly, the H/L ratio of the broiler from the BaP group was always higher ($p<0.05$) than those of the control and Ns commencing from day 7 until 21. Likewise, the H/L ratio of the BaP + Ns group was higher ($p<0.05$) than the control and Ns at day 14. On days 7 and 14, the TP of broilers in the BaP and BaP+ Ns groups were lower ($p<0.05$) than those of the control and Ns groups. This trend continued to be lowest ($p<0.05$) in the BaP group but was comparable to BaP + Ns group until day 21. The A/G ratio of the BaP group was higher ($p<0.05$) than any other group except that of the BaP + Ns from day 7 until day 21. This ratio was higher in the BaP + Ns group from control and Ns groups only at day 7.

Table 5 shows the weight and indices of the spleen, bursa and thymus of broilers during the experimental period. The splenic weight of broilers from the BaP group was always lower ($p<0.05$) than those of control and Ns groups at days 14 and 21. However, that of the BaP + Ns was only lower ($p<0.05$) than control at day 14 only and with that of the Ns at days 14 and 21. Splenic index of the BaP group was always lower ($p<0.05$) than those of control and Ns groups at days 7 n 14. However, the splenic index of the BaP + Ns was only lower ($p<0.05$) than those of the control and Ns at day 7.

The bursal and thymic weights and their indices of broilers in the BaP group was lower ($p<0.05$) than those of control and Ns groups at days 7 and was the lowest ($p<0.05$) compared to all other groups at day 14. However, the bursal weight and index in the BaP + Ns was lower ($p<0.05$) than that of the control at day 7 but with the Ns group, significant difference was on days 7 and 14. Likewise, at days 7 and 14, the thymic weight and index of the BaP + Ns group was lower ($p<0.05$) than that of the control but remained comparable to the control group at day 14.

Table 6 shows the ND-HI titer of broilers throughout the experimental period. While no significant changes were seen at Day 0, commencing from days 14 to 35, the ND-HI titer of the birds from the BaP was always lower ($p<0.05$) compared to that of the control and Ns groups (Table 6). The ND-HI titer of the BaP + Ns group was only comparable to the BaP group at day 14 and to the control and Ns groups at day 35.

Table 7 shows the PHA response of broilers during the experimental period. No significant differences were seen during the pre-injection stage. Post-inject response was always lowest ($p<0.05$) in the BaP group at days 14 and 21. This response in the BaP + Ns group was lower ($p<0.05$) than that of the control at day 14 and to that of

Table 1. The body weight (g) of broilers during the experimental period (mean ± SD)*.

Groups	Days p. i.				
	0	7	14	21	35
Control	44±2.48[a]	180±7.50[a]	509±18.0[a]	971±19.4[a]	1981±43.6[a]
Ns	42±2.58[a]	177±6.50[a]	512±44.4[a]	981±40.7[a]	1990±24.0[a]
BaP	43±1.67[a]	150±10.6[b]	429±30.0[b]	844±36.4[b]	1853±48.1[b]
BaP + Ns	41±1.30[a]	159±7.32[b]	468±24.0[ab]	922±58.0[a]	1929±34.2[a]

*: Mean ± SD (n=8 birds per group); [a, b] Values bearing similar superscript within column do not differ at ($p<0.05$).

the Ns group at day 14 and 21. The PHA response was the lowest ($p<0.05$) in the BaP group at days 14 and 21. The response of the BaP + Ns group was lower than those of the control and Ns groups at day 0. However, on day 21, the lower ($p<0.05$) PHA response in the BaP + Ns group than that of the Ns group was comparable to those of the control and BaP groups.

Table 8 shows the CCA activity in the broilers during the experimental period. The BaP group exhibited the greatest sluggishness ($p<0.05$) compared to control and Ns groups in removing carbon at almost all instances and being comparable to the BaP + Ns group at 7 and 15 minutes (day 14) and 15 minutes (day 35). The CCA activity in the BaP + Ns group was lower ($p<0.05$) than those of the control and Ns group at 3 and 7 minutes (day 14) and 7 minutes (day 21).

Histopathology

While normal microscopic appearance was observed in the spleen, bursa and thymus of chickens from the control group (Figs. 1A, 2A, 3A), those of the BaP groups exhibited significant lesions. Changes in the spleen (Fig. 1C) and bursa (Fig. 2C) were comprised of depletion of cells within the white pulp and medulla,

Table 2. The body weight gain (g) of broilers during the experimental period (mean ± SD)*.

Groups	Days p. i.		
	1-21	21-35	1-35
Control	926±18.8[a]	1010±56.0[a]	1936±42.6[a]
Ns	939±41.0[a]	1008±62.1[a]	1947±26.5[a]
BaP	800±36.2[b]	1009±29.6[a]	1810±48.5[b]
BaP + Ns	880±58.4[a]	1004±84.0[a]	1884±38.2[a]

*: Mean ± SD (n=8 birds per group); [a, b] Values bearing similar superscript within column do not differ at ($p<0.05$).

Table 3. The FI (g) and FCR of broilers during the experimental period (Mean ± SD)*.

Groups	Days p. i.					
	1-21		21-35		1-35	
	FI	FCR	FI	FCR	FI	FCR
Control	1082±58[a]	1.168±0.025[a]	2125±136[a]	2.103±0.117[a]	3207±196[a]	1.656±0.038[a]
Ns	1085±52[a]	1.155±0.050[a]	2168±138[a]	2.150±0.133[ac]	3253±192[ab]	1.670±0.023[a]
BaP	1028±65[b]	1.285±0.059[b]	2230±195[b]	2.210±0.065[b]	3258±231[b]	1.800±0.049[b]
BaP+ Ns	1073±61[a]	1.219±0.082[a]	2202±184[ab]	2.193±0.185[bc]	3275±218[b]	1.738±0.035[b]

*: Mean ± SD (n=8 birds per group); [a, b, c] Values bearing similar superscript within column do not differ at ($p<0.05$).

Table 4. The total WBC counts and H/L ratio of broilers during the experimental period (mean ± SD)*.

Parameters	Groups	Days p. i.			
		7	14	21	35
Total WBCs (x10^9/L)	Control	18.9±0.336[a]	20.9±0.608[ab]	21.4±1.401[ab]	22.1±1.384[a]
	Ns	19.0±1.005[a]	21.5±1.412[a]	21.5±1.479[a]	21.8±0.952[a]
	BaP	15.4±0.771[b]	17.8±1.340[c]	19.3±0.834[b]	20.7±0.780[a]
	BaP + Ns	16.0±0.750[b]	19.1±0.650[bc]	20.3±1.042[ab]	20.8±0.841[a]
H/L (Ratio)	Control	0.448±0.058[a]	0.423±0.048[a]	0.402±0.020[a]	0.437±0.063[a]
	Ns	0.457±0.062[a]	0.397±0.057[a]	0.398±0.020[a]	0.409±0.058[a]
	BaP	0.667±0.093[b]	0.576±0.054[b]	0.513±0.042[b]	0.451±0.048[a]
	BaP + Ns	0.614±0.148[ab]	0.522±0.016[b]	0.440±0.031[a]	0.413±0.037[a]
TP (g/L)	Control	31.82±0.872[a]	31.96±0.814[a]	33.18±0.535[a]	33.50±1.111[a]
	Ns	31.52±0.925[a]	32.34±0.415[a]	33.30±1.048[a]	33.60±1.298[a]
	BaP	27.60±1.088[b]	28.08±1.346[b]	29.88±0.993[b]	32.82±0.606[a]
	BaP + Ns	28.28±0.749[b]	29.64±0.776[b]	31.82±1.688[ab]	32.98±1.294[a]
A/G	Control	0.820±0.048[a]	0.842±0.054[a]	0.833±0.034[a]	0.812±0.062[a]
	Ns	0.847±0.038[a]	0.879±0.082[a]	0.845±0.042[a]	0.839±0.050[a]
	BaP	0.642±0.051[b]	0.698±0.043[b]	0.743±0.029[b]	0.790±0.019[a]
	BaP + Ns	0.666±0.065[b]	0.787±0.066[ab]	0.798±0.047[ab]	0.822±0.059[a]

* Mean ± SD (n=8 birds per group); [a, b, c] Values bearing similar superscript within column do not differ at ($p<0.05$).

respectively. In the thymus (Fig. 3C), apart from a depletion of cells, degenerating and necrotic Hassall's corpuscle was also seen. BaP with Ns group were showed few changes such as slight depletion of lymphocytes within the white pulp, cortex and medulla of the spleen, thymus and bursa respectively. Also there was a mild infiltration by mononuclear cells, especially around the blood vessels, (Figs. 1D, 2D, 3D) as compared with BaP alone.

Table 5. The effect of BaP exposure on lymphoid organ weight and index of broilers during the experimental period (mean ± SD)*.

Parameters	Groups	Days p. i. 14	21	35
Spleen weight (g)	Control	0.394±0.018^{a}	0.948±0.087^{ab}	2.549±0.141^{a}
	Ns	0.410±0.043^{a}	1.046±0.137^{a}	2.605±0.119^{a}
	BaP	0.280±0.035^{b}	0.605±0.103^{c}	2.381±0.183^{a}
	BaP+Ns	0.320±0.055^{b}	0.821±0.083^{b}	2.540±0.067^{a}
Spleen index* Mean x10^{3}	Control	0.774±0.020^{ab}	0.975±0.071^{a}	1.286±0.054^{a}
	Ns	0.799±0.026^{a}	1.064±0.122^{a}	1.308±0.045^{a}
	BaP	0.668±0.040^{b}	0.714±0.089^{b}	1.284±0.082^{a}
	BaP+Ns	0.682±0.111^{b}	0.893±0.102^{a}	1.309±0.049^{a}
Bursa weight (g)	Control	0.791±0.045^{a}	1.722±0.087^{a}	3.539±0.139^{a}
	Ns	0.764±0.063^{a}	1.817±0.091^{a}	3.528±0.154^{a}
	BaP	0.491±0.065^{b}	1.213±0.111^{c}	3.203±0.113^{b}
	BaP+Ns	0.566±0.074^{b}	1.541±0.097^{b}	3.326±0.179^{ab}
Bursa index* Mean x10^{3}	Control	1.552±0.039^{a}	1.773±0.065^{ab}	1.786±0.040^{a}
	Ns	1.494±0.046^{a}	1.851±0.031^{a}	1.772±0.059^{a}
	BaP	1.140±0.082^{b}	1.435±0.080^{c}	1.729±0.071^{a}
	BaP+Ns	1.209±0.152^{b}	1.676±0.121^{b}	1.727±0.09^{a}
Thymus weight (g)	Control	1.675±0.069^{a}	2.822±0.204^{ab}	5.227±0.194^{a}
	Ns	1.701±0.069^{a}	2.996±0.260^{a}	5.308±0.199^{a}
	BaP	1.085±0.151^{b}	2.070±0.228^{c}	4.958±0.116^{a}
	BaP+Ns	1.262±0.186^{b}	2.477±0.139^{b}	5.113±0.409^{a}
Thymic index* Mean x10^{3}	Control	3.291±0.070^{a}	2.904±0.154^{ab}	2.638±0.080^{a}
	Ns	3.337±0.251^{a}	3.028±0.165^{a}	2.667±0.090^{a}
	BaP	2.523±0.257^{b}	2.450±0.201^{c}	2.676±0.053^{a}
	BaP+Ns	2.684±0.302^{b}	2.689±0.112^{bc}	2.653±0.186^{a}

*: Mean ± SD (n=8 birds per group); $^{a, b, c}$ Values bearing similar superscript within column do not differ at ($p<0.05$); *: Lymphoid organ index, organ weight (g)/total weight (g) x1000.

Table 6. The ND-HI antibody titers of broilers during the experimental period (log2, mean ± SD)*.

Groups	Days p.i. 0	14	21	35
Control	4.0±0.707^{a}	4.6±0.516^{a}	5.9±0.567a	6.9±0.567^{a}
Ns	4.2±0.836^{a}	4.6±0.516^{a}	5.9±0.316a	7.0±0.471^{a}
BaP	4.0±0.707^{a}	3.4±0.699^{b}	4.1±0.567c	5.5±0.527^{b}
BaP+Ns	4.0±1.000^{a}	3.6±0.699^{b}	5.2±0.421b	6.5±0.527^{a}

*: Mean ± SD (n=8 birds per group); $^{a, b, c}$ Values bearing similar superscript within column do not differ at ($p<0.05$).

Discussion

Immunotoxicity is a challenging area of toxicology since the immune system is regulated by many external factors and feedback mechanisms (Descortes, 2000). Heightened sensitivity of the immune system, to even low levels of xenobiotics, makes it a reliable system to study the effects of immunotoxicity (Sharma and Reddy, 1987). It is remarkable to observe a growth stunting effect of BaP at 15 mg/kg BW on broilers performance indices (BW, BWG, FI and FCR). To our knowledge, no

Table 7. The effect of BaP exposure on the Phytohaemagglutinin (PHA) skin test (pre and post-injection) of broilers during the experimental period (mean ± SD)*.

Parameters	Groups	Days p. i. 14	21	35
Post-injection	Control	1.512±0.034^{a}	1.734±0.118^{a}	1.890±0.056^{a}
	Ns	1.552±0.039^{a}	1.738±0.109^{a}	1.902±0.069^{a}
	BaP	1.194±0.042^{c}	1.512±0.068^{b}	1.846±0.118^{a}
	BaP+Ns	1.356±0.126^{b}	1.604±0.115^{ab}	1.938±0.044^{a}
^{A}PHA response	Control	0.736±0.025^{a}	0.850±0.097^{ab}	0.950±0.068^{a}
	Ns	0.776±0.023^{a}	0.860±0.088^{a}	0.968±0.078^{a}
	BaP	0.442±0.049^{c}	0.580±0.057^{c}	0.878±0.036^{a}
	BaP+Ns	0.574±0.053^{b}	0.710±0.064^{bc}	0.946±0.042^{a}

* Mean ± SD (n=8 birds per group); $^{a, b, c}$ Values bearing similar superscript within column do not differ at ($p<0.05$); ^{A}PHA=(PHA response, patagia post-injection) - (PHA response, patagia pre-injection).

Table 8. The effect of BaP exposure on phagocytic activity of broilers during the experiment period (mean ± SD)*.

Parameter	Groups	Minutes	Days p. i. 14	21	35
	Control		38.1±1.699^{a}	38.9±4.551^{a}	33.5±1.883^{ab}
	Ns		35.8±2.320^{a}	36.5±2.139^{a}	30.7±3.050^{a}
	BaP	3	61.9±5.223^{c}	51.1±2.772^{b}	39.8±4.334^{b}
	BaP+Ns		48.8±6.202^{b}	40.9±4.974^{a}	31.2±4.608^{a}
CCA	Control		26.6±3.020^{a}	22.8±1.922^{a}	24.8±1.515^{ab}
	Ns		24.3±5.267^{a}	21.8±1.595^{a}	23.0±0.954^{a}
	BaP	7	37.7±3.090^{b}	34.0±1.975^{c}	29.1±4.817^{b}
	BaP+Ns		35.2±4.061^{b}	30.0±2.846^{b}	22.7±0.866^{a}
	Control		16.0±2.670^{a}	15.1±1.885^{a}	13.8±1.539^{a}
	Ns		14.8±2.921^{a}	14.0±2.562^{a}	10.9±3.083^{a}
	BaP	15	27.2±6.901^{b}	22.8±2.635^{b}	19.1±3.335^{b}
	BaP+Ns		22.2±3.788^{ab}	13.7±3.678^{a}	15.4±2.088^{ab}

*: Mean ± SD (n=8 birds per group); $^{a, b, c}$ Values bearing similar superscript within column do not differ at ($p<0.05$).

similar report has been recognized in broilers, though De Jong et al. (1999) reported this in rats. The dose of BaP used by De Jong (1999) that showed a toxic effect was much lower than that ultilised in our study indicating higher resistance of broilers to BaP as compared to mammals. The Ns supplementation improves broilers performance after 14 day by reducing the effects of BaP toxicity as evidenced by an increase in BW, BWG and FCR in comparison with BaP alone. This increase in broiler's performance given Ns seen in our study is similar to that reported by Ashayerizadeh et al. (2009) and is believed to be attributed to the antioxidant promoting (Ramadan, 2007) and/or immunomodulating (Soliman et al., 1999) activities of Ns.

White blood cells are the effector cells of immune responses and have been assessed in many immunotoxicological studies of avian wildlife (Grasman, 2002). The normal lower range limits of selected haemogram parameters (Bartholomew et al., 1998) in the BaP signified a possible episode of weakened defense. This negative interaction may have disastrous consequences if an infection sets in during this period. As will be discussed later, such phenomenon coupled with suppressed antibody production will inevitably produce a destabilized immune system. Suppression of WBCs may arise from a direct effect of BaP on the haemopoietic tissues (Fig. 1C) leading to an increase in the rate of WBC destruction in the circulation (Leighton et al., 1985) and induction of apoptosis in stromal/feeder cells, impairing mononuclear cell function (Romero et al., 1997). We believe that activation of aryl hydrocarbon receptors (de Oliveira et al., 2007) during metabolism of the instilled BaP orchestrated oxidative stress (Chen et al., 2004) that consequently induced haematopoietic

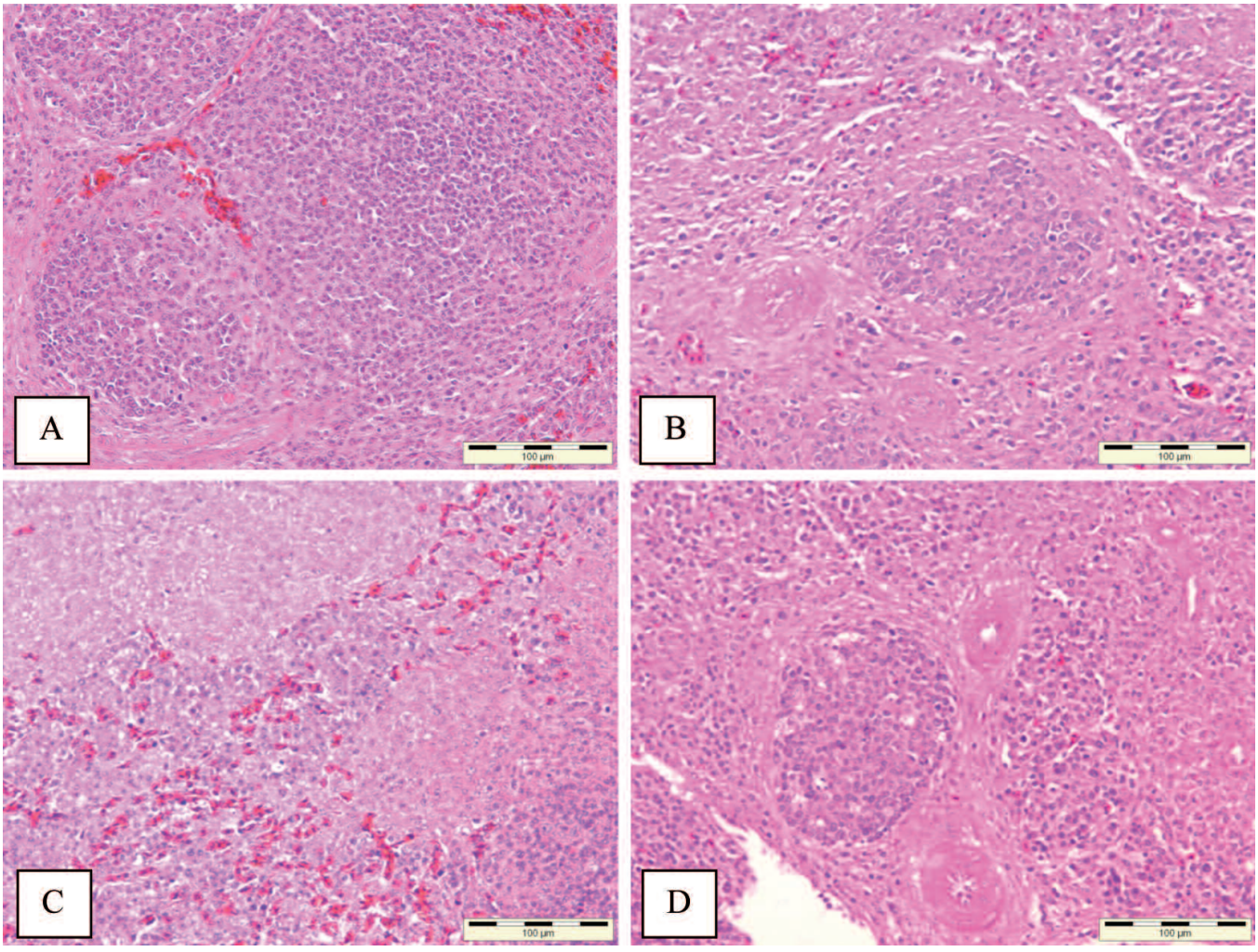

Fig. 1. Photomicrograph of the spleen of chickens necropsied at 21 day p.i. **A.** The histology of control group within normal limits. **B.** Similarly, a normal histology was also seen in the Ns group. **C.** However, apart from congestion, that of the BaP group exhibited severe depletion of lymphocytes within that region. **D.** Only slight depletion of lymphocytes with mild infiltration of mononuclear cells is seen the BaP+Ns group. H&E. Bar: 100 μm.

impairment (Pang et al., 2008). This series of events was manifested as much lower haemogram values in chickens from the BaP group.

Assessment of plasma or serum protein concentrations can provide important data on health and physiological status, including immune and inflammation responses (Grasman, 2002). Impaired protein synthesis due to BaP toxicity (Jee et al., 2006) leading to decreased albumin (Walseth et al., 1982) concentration has indirectly explained the lower TP concentration and A/G ratio in broilers from the BaP group. This also shows that the administered BaP had a systemic effect despite being given via the i.t. route (Sun et al., 1982). Nevertheless, the waning of the immunosuppression as evidenced by the return of normal values over time is undoubtedly suggestive of the half-life of BaP (Sun et al., 1982). The 30 day lapse between the last instillation and the end of the experiment has left enough time for the remaining administered BaP to be excreted. In this experiment it was shown that supplementation of Ns seeds slightly alleviated the adverse effects of BaP at 7 days post-feeding. It is likely that the antioxidant properties of Ns seed protected the haematopoietic tissues against oxidative stress (Suboh et al., 2004), and ameliorated the reduction in the WBC (Meral and Kanter, 2003). The comparable concentration of TP and A/G ratio of broilers in the Ns and BaP + Ns group to the control signified hepato-protective effects of Ns against BaP (Nagi et al., 1999; El-Dakhakhny et al., 2000). Alternatively, this shows that Ns administration is efficacious in preventing broilers from oxidative stress

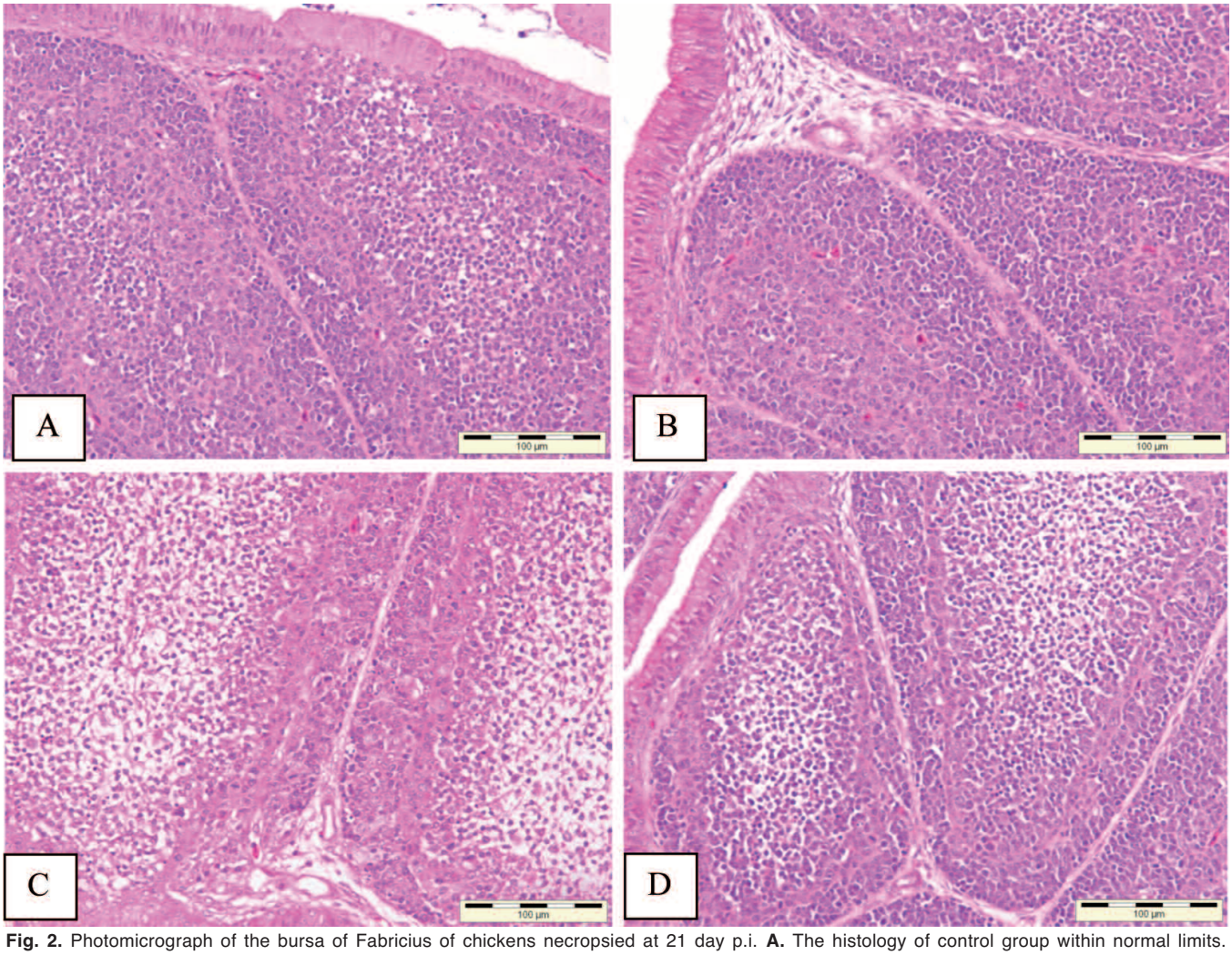

Fig. 2. Photomicrograph of the bursa of Fabricius of chickens necropsied at 21 day p.i. **A.** The histology of control group within normal limits. **B.** Likewise, a normal histology was also seen in the Ns group. **C.** The bursa in the BaP group demonstrated degeneration and necrosis of the lymphocytes in the medullary area. Note interfollicular edema mixed with mononuclear phagocytic cells. **D.** The histology of the bursa in the BaP + Ns group was appeared normal H&E. Bar: 100 μm.

and immunosuppression (Ashayerizadeh et al., (2009).

Assessment of antibody titers to ND is still commonly done in many laboratories by HI test (Heckert and Nagy, 1999). Immunotoxicity (Trust et al., 1994; Ross et al., 1996) led to a decrease of ND-HI titers in the BaP group, especially at days 14 and 21. At these instances, the titers barely surpassed the designated protective mark (Takada and Kida, 1996; OIE, 2008). Likewise, the clear lack of splenic-derived secondary immune response following day 7 post-immunization (Mast and Goddeeris, 1999) further denotes immunotoxicity in the BaP group. The findings in our study reinforced the importance of avian spleen in antibody production (Jeurisson, 1991) and the damage to splenic anatomy by BaP (Fig. 1C), which may have affected antibody production. As discussed earlier, the BaP-exposed broilers stand a high risk of succumbing to an infection during this period, with a disastrous outcome. At these instances, the titers in the Ns treated group was higher than the BaP alone after day 14 was related to absence of lesions in the spleen and bursa in these groups. This has led to the ability of broilers in this group to elicit adequate immune response (Jeurisson, 1991).

The slower spatial peak of PHA skin response and resolution of the inflammation in the BaP group is possibly a BaP-derived inhibition on both T- and B-cell-mediated immune responses (Trust et al., 1994). Elimination of T lymphocyte function by irradiation or immunosuppressive drugs decreases the PHA skin response by 50-60% in birds (Schrank et al., 1990; Grasman and Scanlon, 1995). As mentioned earlier, our

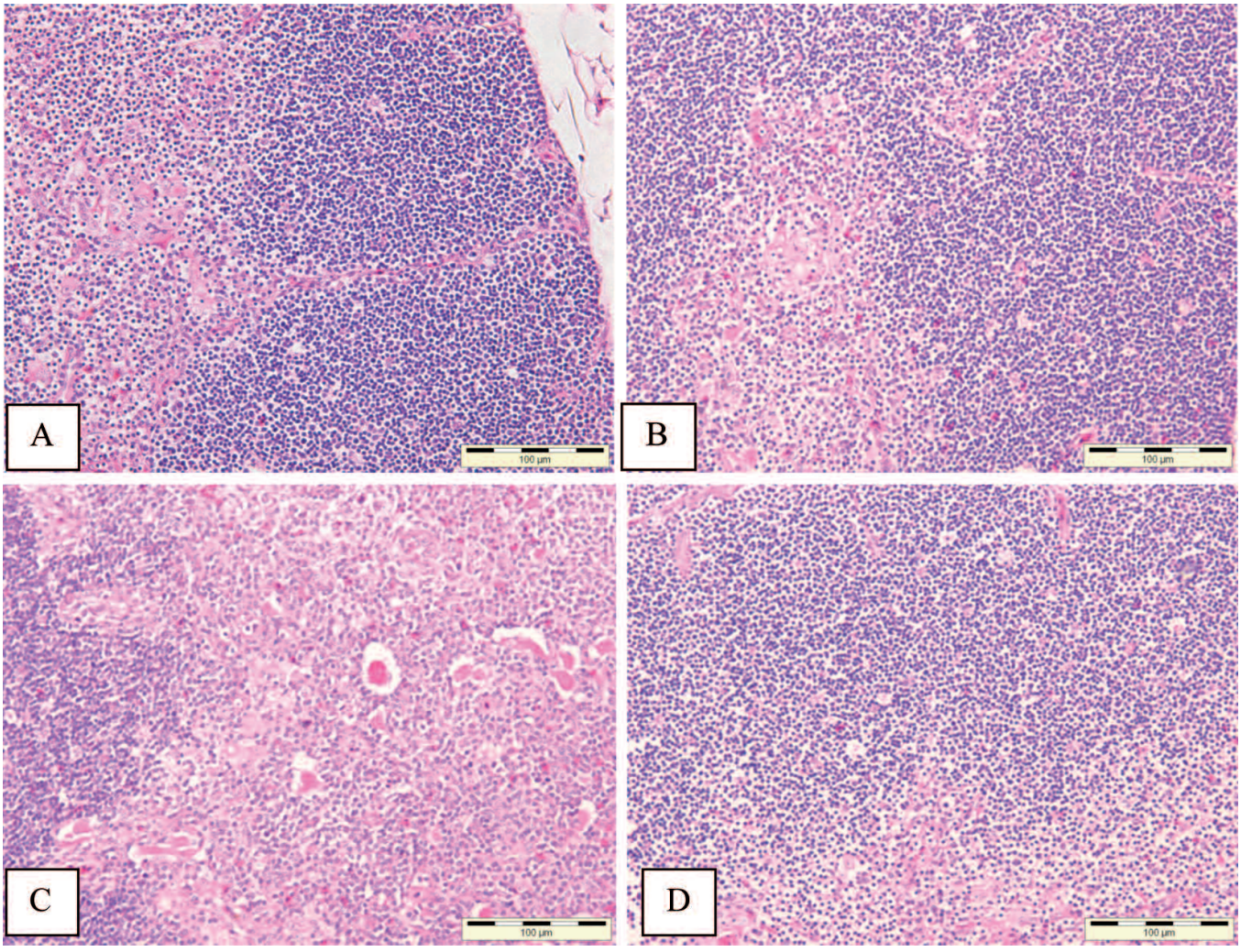

Fig. 3. Photomicrograph of the thymus of chickens necropsied at 21 days p.i. **A.** The histology of control group within normal limits. **B.** A normal histology was also seen in the Ns group. **C.** Thinning of the cortex, Hassall's corpuscle degeneration and necrosis along with focal vacuolation and destruction of lymphocyte existed in this group in the BaP group. **D.** A less severe lesion as described in the BaP group is seen in the BaP group. H&E. Bar: 100 μm.

results showed that BaP might have suppressed the migration of the cell to the site of the injection, indicating immunotoxicity. Indeed, potent immunosuppressive PAHs such as BaP were shown to inhibit murine T and B cell proliferation and alter T cell-related cytokine production and B cell-mediated antibody production (Blanton et al., 1988; Pallardy et al., 1989). They also suppress mitogenesis of human T lymphocytes (Mudzinski, 1993) and alter B cell lymphopoiesis by triggering pre-B lymphocyte apoptosis (Yamaguchi et al., 1997). In the study presented here, evidence for these mechanisms is partially depicted in lesions seen in the lymphoid organs (Figs. 1c, 2c, 3c).

In this study, evidence for a reduced effect of BaP was detected in the Ns treated group, due to partially depicted lesions seen in the lymphoid organs of this group (fewer morphological alterations). This may be related to the anti-inflammatory properties exerted by Ns (Haq et al., 1995) in normalizing the elevated cytokine profiles (Hajhashaemi et al., 2004) thus resulting in a potential suppression of inflammation through inhibition of leukotrienes constitutes (Mansour and Tornhamre, 2004). Ns constituent has been shown to have the potential activity to normalize elevated levels of macrophage derived inflammatory mediators (El-Mahmoudy et al., 2005). As seen in the present study, active ingredients within Ns showed elicited immunomodulatory properties via augmentation of the T cell- and natural killer cell mediated immune responses activity (Padhye et al., 2008) leading to better CCA activity.

Under normal conditions the injected carbon is supposed to be engulfed by the cells of the mononuclear phagocytic system, including the circulating monocytes and fixed tissue macrophages (Qureshi et al., 2000). Our results indicated defective phagocytic cell function in broiler chickens exposed to BaP that was similar to that seen in fish (Weeks et al., 1986). The functional properties characterizing macrophagic cells, such as endocytosis and phagocytosis (van Grevenynghe, 2003) are likely to have been altered by BaP exposure, which in turn weakened their function, and this exhaustion is manifested as a delayed CCA in the BaP group. On the other hand, Ns treatment alleviates the adverse effects of BaP, indicated by less carbon density in the blood, and high engulfing activities; this may be attributed to the enhancement of production of IL-3 by lymphocytes, which IL-3 has a stimulatory effect on macrophages (Haq et al., 1995). In accordance with previous study (Fararh et al., 2004) and proven here again, oral administration of Ns can significantly induced an elevation in the phagocytic activity in clearing particles as demonstrated by the CCA activity in broilers receiving Ns with or without BaP.

Our results showed that Ns treatment protected the broilers against BaP toxicity and reduced the effects on lymphoid organs, resulting in an improvement of immunity status due to their action on free radical balance, which is important in signaling and regulating the immune system. The lymphoid organ indices are useful indicators of toxicant effect (Sellers et al., 2007) and on the ability of the animals to tolerate the toxicant stressors (Eerola et al., 1987) or provide lymphoid cells during an immune response (Heckert et al., 2002). Even with the increase in lymphoid organ weight over time, the reduction in lymphoid indices was due to a much higher weight gain. Such a discrepancy led to a lower lymphoid index, although the absolute volume or weight of the organs increased. Furthermore, the lower lymphoid organ weight and associated lesions in the BaP group signified toxicity (Bailey et al., 2004), which is invoked by the instilled BaP (Kontoni et al., 1999). Alternatively, such decrement of organ indices may be attributed to the atrophy of organs, leading to a much smaller size as well as their ratios as seen in the BaP group (Lavoie and Grasman, 2007). Indisputably, the atrophied lymphoid organs seen in the BaP group have hampered lymphocyte development and function (Briggs et al., 1996), explaining the low levels of HI titers and slow CCA response in this group. This signified that Ns treatments has protected the broilers against BaP immunotoxicity and impaired phagocytic function.

In conclusion, these data, together with the histological findings, indicated that exposure to BaP impairs the growth performance, response of the immune system and phagocytic activity of broiler chickens. Although immunosuppression exerted by BaP in this study appeared as transient, it is long enough to result devastating consequences once an infection sets in. Supplementation of Ns enhanced the broiler performance and immune-modulatory properties. Further studies are urgently required to explore the exact effects of Ns on immunotoxicity and immunosuppressive activity of BaP, which is likely to substantially improve the immunotherapeutic application of Ns in clinical settings.

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References

Ali B.H. and Blunden G. (2003). Pharmacological and toxicological properties of *Nigella sativa*. Phytother. Res. 17, 299-305.

Allan W. and Gough R. (1974). A standard haemagglutination inhibition test for Newcastle disease. I. A comparison of macro and micro methods. Vet. Rec. 95, 120-123.

Amagase H., Sun B. and Borek C. (2009). *Lycium barbarum* (goji) juice improves *in vivo* antioxidant biomarkers in serum of healthy adults. Nutr. Res. 29, 19-25.

Ashayerizadeh O., Dastar B., Shargh M.S., Ashayerizadeh A., Rahmatnejad E. and Hossaini S.M. (2009). Use of garlic (*Allium sativaum*), black cumin seeds (*Nigella sativa* L.) and wild mint (*Mentha longifolia*) in broiler chickens diets. J. Anim. Vet. Adv. 8, 1860-1863.

Bailey S., Zidell R. and Perry R. (2004). Relationships between organ

weight and body/brain weight in the rat: what is the best analytical endpoint? Toxicol. Pathol. 32, 448-466.

Bartholomew A., Latshaw D. and Swane D. (1998). Changes in blood chemistry, hematology, and histology caused by a selenium/vitamin E deficiency and recovery in chicks. Biol. Trace Elem. Res. 62, 7-16.

Blanton R.H., Myers M.J. and Bick P.H. (1988). Modulation of immunocompetent cell populations by benzopyrene. Toxicol. Appl. Pharmacol. 93, 267-274.

Boström C.-E., Gerde P., Hanberg A., Jernström B., Johansson C., Kyrklund T., Rannug A., Törnqvist M., Victorin K. and Westerholm R. (2002). Cancer risk assessment, indicators, and guidelines for polycyclic aromatic hydrocarbons in the ambient air. Environ. Health Perspect. 110, 451-488.

Briggs K.T., Yoshida S.H. and Gershwin M.E. (1996). The influence of petrochemicals and stress on the immune system of seabirds. Regul. Toxicol. Pharmacol. 23, 145-155.

Carlson E.A., Li Y. and Zelikoff J.T. (2004). Suppressive effects of benzo[a]pyrene upon fish immune function: Evolutionarily conserved cellular mechanisms of immunotoxicity. Mar. Environ. Res. 58, 731-734.

Chen Z.H., Hurh Y.J., Na, H.K., Kim J.H., Chun Y.J., Kim D.H., Kang K.S., Cho M.H. and Surh Y.J. (2004). Resveratrol inhibits TCDD-induced expression of CYP1A1 and CYP1B1 and catechol estrogen-mediated oxidative DNA damage in cultured human mammary epithelial cells. Carcinogenesis 25, 2005-2013.

Cheng S. and Lamont S.J. (1988). Genetic analysis of immunocompetence measures in a White Leghorn chicken line. Poult. Sci. 67, 989-995.

Choi E.M., Koo S.J. and Hwang J.K. (2004). Immune cell stimulating activity of mucopolysaccharide isolated from yam (*Dioscorea batatas*). J. Ethnopharmacol. 91, 1-6.

De Buck S.S., Bouche F.B., Brandenburger A. and Muller C.P. (2005). Modulation of the metabolism and adverse effects of benzo[a]pyrene by a specific antibody: a novel host factor in environmental carcinogenesis? Carcinogenesis 26, 835-844.

De Jong W.H., Kroese E. D., Vos G.J. and Van Loveren H. (1999). Detection of immunotoxicity of benzo[a]pyrene in a subacute toxicity study after oral exposure in rats. Toxicol. Sci. 50, 214-220.

de Oliveira S.K., Hoffmeister M., Gambaryan S., Muller-Esterl W., Guimaraes J.A. and Smolenski A.P. (2007). Phosphodiesterase 2A forms a complex with the co- chaperone XAP2 and regulates nuclear translocation of the aryl hydrocarbon receptor. J. Biol. Chem. 282, 13656-13663.

DerMarderosian A., Liberti L., Beutler J., Grauds C. and Tatro D.S (2005). The review of natural products. 4th Edn. Facts and Comparisions. Lipincott Williams & Wilkins.

Descotes J. (2000). Integrating immunotoxicity with effects on other biological systems in preclinical safety evaluation: a perspective. Toxicology 142, 157-160.

Eerola E. Veromaa T. and Toivanen P. (1987). Special features in the structural organization of the avian lymphoid system. In: Avian Immunology: Basis and practice. Toivanen A. and Toivanen P. (eds). Boca Raton: FL CRC Press. pp 9-22.

El-Dakhakhnya M., Madia N.J., Lembertb N. and Ammon H.P.T. (2002). *Nigella sativa* oil, nigellone and derived thymoquinone inhibit synthesis of 5-lipoxygenase products in polymorphonuclear leukocytes from rats. J. Ethnopharmacol. 81, 161-164.

El-Dakhakhny M., Mady N.I. and Halim M.A. (2000). *Nigella sativa* L. oil protects against induced hepatotoxicity and improves serum lipid profile in rats. Arzneimittelforschung 50, 832-836.

El-Mahmoudy A., Shimizu Y., Shiina T., Matsuyama H., Nikami H. and Takewaki T. (2005). Macrophage-derived cytokine and nitric oxide profiles in type I and type II diabetes mellitus: effect of thymoquinone. Acta Diabetol 42, 23-30.

Fararh K.M., Atoji Y., Shimizu Y., Shiina T., Nikami H. and Takewaki T. (2004). Mechanisms of the hypoglycaemic and immunopotentiating effects of *Nigella sativa* L. oil in streptozotocininduced diabetic hamsters. Res. Vet. Sci. 77, 123-129.

Grasman K.A. (2002). Assessing immunological function in toxicological studies of avian wildlife. Integr. Comp. Biol. 42, 34-42.

Grasman K.A. and Scanlon F.P. (1995). Effects of acute lead ingestion and diet on antibody and T-cell-mediated immunity in Japanese quail. Arch. Environ. Contam. Toxicol. 28, 161-167.

Gupta R.P. and Abou-Donia M.B. (1998). Cytochrome P450 enzymes in chickens: characteristics and induction by xenobiotics. Comp. Biochem. Physiol. 121, 73-83.

Hajhashemi V., Ghannadi A. and Jafarabadi H. (2004). Black cumin seed essential oil, as a potent analgesic and anti-inflammatory drug. Phytother. Res. 18, 195-199.

Haq A., Abdullatif M., Lobo P.I., Khabar K.S., Sheth K.V. and Al-Sedairy S.T. (1995). *Nigella sativa*: effect on human lymphocytes and polymorphonuclear leukocyte phagocytic activity. Immunopharmacology 30, 147-155.

Heckert R., Estevez I., Russek-Cohen E. and Pettit-Riley R. (2002). Effects of density and perch availability on the immune status of broilers. Poult. Sci. 81, 451-457.

Heckert R.A. and Nagy E. (1999). Evaluation of the haemagglutination-inhibition assay using a baculovirus-expressed haemagglutinin-neuraminidase protein for detection of Newcastle disease virus antibodies. J. Vet. Diagn. Invest. 11, 99-102.

Heller E.D., Leitner G., Friedman A., Uni Z., Gutman M. and Cahaner A. (1992). Immunological parameters in meat-type chicken lines divergently selected by antibody response to Escherichia coli vaccination. Vet. Immunol. Immunopathol. 34, 159-172.

Jee J.H., Park K.H., Keum Y.H. and Kang J.C. (2006). Effects of 7,12-dimethylbenz[a]anthracene on growth and haematological parameters in Korean rockfish, *Sebastes schlegeli* (Hilgendorf). Aquacult. Res. 37, 431-442.

Jeurissen S. (1991). Structure and function of the chicken spleen. Res. Immunol. 142, 352-355.

Khalife K.H. and Lupidi G. (2007). Nonenzymatic reduction of thymoquinone in physiological conditions. Free Radic. Res. 41, 153-161.

Kontoni N., Hashimoto H., Sessler D., Yasuda T., Ebina T., Muraoka M. and Mastsuki A. (1999). Expression of genes for pro-inflammatory cytokines in alveolar macrophages during propofol and isoflurane anesthesia. Anesth. Analg. 89, 1250-1256.

Latif I.K., Karim A.J., Zuki A.B.Z., Zamri-Saad M., Niu J.P. and Noordin M.M. (2009). Respiratory macrophage activity and pulmonary morphology following exposure to benzo[a]pyrene in broilers. Online J. Vet. Res. 13, 128-135.

Latif I.K., Karim A.J., Zuki A.B.Z., Zamri-Saad M., Niu J.P. and Noordin, M.M. (2010). Pulmonary modulation of benzo[a]pyrene-induced hemato- and hepatotoxicity in broilers. Poult. Sci. 89, 1379-1388.

Lavoie E.T. and Grasman K.A. (2007). Effects of *in ovo* exposure to PCBs 126 and 77 on mortality, deformities and post-hatch immune function in chickens. J. Toxicol. Environ. Health Part A 70, 547-558.

Leighton F. (1985). Morphological lesions in red blood cells from herring

gulls and Atlantic puffins ingesting Prudhoe Bay crude oil. Vet. Pathol. 22, 393-402.

Lin J-Y., Lu S., Liou Y.L. and Liou H.L. (2006). Antioxidant and hypolipidaemic effects of a novel yam-boxthorn noodle in an *in vivo* murine model. Food Chem. 94, 377-384.

Liu R. and Sun J. (2003). Antiproliferative activity of apples is not due to phenolic-induced hydrogen peroxide formation. J. Agric. Food Chem. 51, 1718-1723.

Mansour M. and Tornhamre S. (2004). Inhibition of 5-lipoxygenase and leukotriene C4 synthase in human blood cells by thymoquinone. J. Enzyme Inhib. Med. Chem. 19, 431-436.

Martin L.B., Han P., Lewittes J., Kuuhlman J.R., Klasing K.C. and Wikelski M. (2006). Phytohaemagglutinin-induced skin swelling in birds, histological support for a classic immunological technique. Funct. Ecol. 20, 290-299.

Mast J. and Goddeeris B.M. (1999). Development of immuno-competence of broiler chickens. Vet. Immunol. Immunopathol. 70, 245-256.

Meral I. and Kanter M. (2003). Effects of *Nigella sativa* L. and *Urtica dioica* L. on selected mineral status and hematological values in CCl4-treated rats. Biol. Trace Elem. Res. 96, 263-270.

Mudzinski S.P. (1993). Effects of benzo[a]pyrene on concavalin A-stimulated human peripheral blood mononuclear cells in vitro, inhibition of proliferation but no effect on parameters related to the G1 phase of the cell cycle. Toxicol. Appl. Pharmacol. 119, 166-174.

Nagi M.N., Alam K., Badary O.A., Al-Shabanah O.A., Al-Sawaf H.A. and Al-Bekairi A.M. (1999). Thymoquinone protects against carbon tetrachloride hepatotoxicity in mice via an antioxidant mechanism. Biochem. Mol. Biol. Int. 47, 153-159.

OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals Chapter 2.3.14. -Newcastle disease 2008; pp576-89.http,// www.oie.int/eng/normes/mmanual/2008/pdf/2.03.14_ NEWCASTLE_DIS.pdf accessed on 17 July 2009

Padhye S., Banerjee S., Ahmad A., Mohammad R. and Sarkar F.H. (2008). From here to eternity - the secret of Pharaohs, Therapeutic potential of black cumin seeds and beyond. Cancer Ther. 6, 495-510.

Pallardy M., House R. and Dean J. (1989). Molecular mechanism of 7,12-dimethylbenzanthracene induced immunosuppression, evidence for action via the interleukin-2 pathway. Mol. Pharmacol. 36, 128-133.

Pang P., Lin Y., Lee Y., Hou H., Hsu S. and Juan S. (2008). Molecular mechanisms of p21 and p27 induction by 3- methylcholanthrene, an aryl-hydrocarbon receptor agonist, involved in anti-proliferation of human umbilical vascular endothelial cells. J. Cell Physiol. 215, 161-171.

Phillips J.D. (1992). Medicinal plants. Biologist 39, 187-191.

Qureshi M.A., Heggen C.L. and Hussain I. (2000). Avian macrophage, Effector functions in health and disease. Dev. Comp. Immunol. 24, 103-119.

Ramadan M.F. (2007). Nutritional value, functional properties and nutraceutical applications of black cumin (*Nigella sativa* L.). Int. J. Food Sci. Tech. 42, 1208-1218.

Romero D., Mounho B., Lauer F., Born J. and Burchiel S. (1997). Depletion of glutathione by benzo[a]pyrene metabolites, ionomycin, thapsigargin, and phorbol myristate in human peripheral blood mononuclear cells. Toxicol. Appl. Pharmacol. 144, 162-169.

Ross P., De Swart R., Addison R., Van Loveren H., Vos J. and Osterhaus A. (1996). Contaminant-induced immunotoxicity in harbour seals, Wildlife at risk? Toxicology 112, 157-169.

Schrank C.S., Cook M.E. and Hansen W.R. (1990). Immune responses of mallard ducks treated with immunosuppressive agents, antibody response to erythrocytes and in vivo response to phyto-haemagglutinin-P. J. Wildl. Dis. 26, 307-315.

Sellers R.S., Morton D., Michael B., Roome N., Johnson J., Yano B., Perry R. and Schafer K. (2007). Society of Toxicologic Pathology Position Paper, Organ weight recommendations for toxicology studies. Toxicol. Pathol. 35, 751-755.

Sharma R.P. and Reddy R.V. (1987). Toxic effects of chemicals on the immune system. In: Handbook of toxicology. Haley T.J. and Berndt W.O. (eds). Hemisphere Publishing Corp. Philadelphia. pp 555-591.

Smits J.E., Bortolotti G.R. and Tella J.L. (1999). Simplifying the phytohaemagglutinin skin testing technique in studies of avian immunocompetence. Funct. Ecol. 13, 567-572.

Soliman A.Z.M., Gahzalah A.A., El-Samra S.H., Atta A.M. and Abdo Z.M.A. (1999). The synergistc effect of either black seeds or garlic with fat on broiler performance and immunity. Egypt J. Nutr. Feeds 2, 603-620.

Suboh S.M., Bilo Y.Y. and Aburjai T.A. (2004). Protective effects of selected medicinal plants against protein degradation, lipid peroxidation and deformability loss of oxidatively stressed human erythrocytes. Phytother. Res. 18, 280-284.

Sun J., Wolff R. and Kanapilly G. (1982). Deposition, retention, and biological fate of inhaled benzo[a]pyrene adsorbed onto ultrafine particles and as a pure aerosol. Toxicol. Appl. Pharmacol. 65, 231-244.

Takada A. and Kida H. (1996). Protective immune response of chickens against Newcastle disease, induced by the intranasal vaccination with inactivated virus. Vet. Microbiol. 50, 17-25.

Trust K., Fairbrother A. and Hooper M. (1994). Effects of 7,12-dimethylbenz[a]anthracene on immune function and mixed-function oxygenase activity in the European starling. Environ. Toxicol. Chem. 13, 821-830.

van Grevenynghe J., Rion S., Le Ferrec E., Le Vee M., Amiot L., Fauchet R. and Fardel O. (2003). Polycyclic aromatic hydrocarbons inhibit differentiation of human monocytes into macrophages. J. Immunol. 170, 2374-2381.

Villasenor-Garcia M.M., Lozoya X., Osuna-Torres L., Viveros-Paredes J.M., Sandoval-Ramirez L. and Puebla-Perez A.M. (2004). Effect of Ginkgo biloba extract EGb 761 on the nonspecific and humoral immune responses in a hypothalamic-pituitary-adrenal axis activation model. Int. Immunopharmacol. 4, 1217-1222.

Walseth F., Toftgard R. and Nilsen O.G. (1982). Phthalate esters I, effects on cytochrome P-450 mediated metabolism in rat liver and lung, serum enzymatic activities and serum protein levels. Arch. Toxicol. 50, 1-10.

Weeks B.A., Warinner J.E., Mason P.L. and Mc Ginnis D.S. (1986). Influence of toxic chemicals on the chemotactic response of fish macrophages. J. Fish Biol. 28, 653-658.

Yamaguchi K., Matulka R., Shneider A., Toselli P., Trombino A., Yang S., Hafer L., Mann K., Tao X.-J., Tilly J., Near R. and Sherr D. (1997). Induction of pre B cell apoptosis by 7,12-dimethyl-benz[a]anthracene in long-term primary murine bone marrow cultures. Toxicol. Appl. Pharmacol. 147, 190-203.

Zhang L., Pan L. and Liu J. (2009). Immunotoxicity effect of benzo[a]pyrene on scallop chlamys farreri. J. Ocean Univ. China 8, 89-94.

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