

Original Research Article

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Immunotoxic Effect of Selenium Following Subacute Exposure in Broilers

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ABSTRACT

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This study was conducted with an objective to determine the immunotoxic effect of sub acute exposure of selenium (Se) in broilers with special reference to the Dinitrofluorobenzene (DNFB) contact skin sensitivity test and pathomorphological alterations in histoarchitecture of lymphoid organs. The chicks were intoxicated orally with sodium selenite @ 2, 6 and 10 ppm respectively in diet, daily for 35 days. The present study exhibited significant depression of cell mediated immunity in Se treated broilers as measured by DNFB skin contact sensitization test. The blood picture revealed dose dependant heterophilia and leukocytopenia due to lymphocytopenia. During subacute Se toxicity, severity of pathological changes of treated broilers was dose dependent. Lymphoid organs of selenium treated broilers revealed severe depletion of lymphocytes from the germinal centre.

Introduction

Poultry industry is designated as major dynamic and rapid growing segment amongst agricultural and livestock sectors in India. Selenium (Se) is required in the diets of mammals and poultry, but can easily be over supplemented due to a narrow range of safety between ideal and toxic concentrations.

Sodium selenate and sodium selenite are used as supplements to poultry and livestock feed to promote growth and prevent selenium deficiency diseases. Selenium can be toxic for all animals, such as invertebrates, fishes, amphibians and reptiles, birds, mammals and

humans depending on the dose and duration of intake and also on its chemical form. Traditionally, Se has been added to poultry diets via inorganic sources, such as sodium selenite (Na_2SeO_3). Selenium in combination with vitamin E is used frequently in poultry for immunomodulation.

The immunomodulatory effect and toxicity of inorganic selenium, such as selenite and selenate, has been heavily studied in animals, aquatic birds and fishes. But till date work on immunotoxicity of Se is scanty. So, present study has designed to demonstrate the immunotoxic effect of selenium following subacute exposure in broilers.

Materials and Methods

Chicken and diet

The experimental investigation was planned to adjudge the toxicopathological effects of sodium selenite on cell mediated immune response in broilers after obtaining approval from Institutional Animal Ethics Committee. Clinically healthy one hundred and twenty, day old, Cobb- 400 broiler chicks of both sexes, weighing 45- 49 g were obtained from a commercial hatchery and were reared on deep litter system of housing using rice husk with provision of artificial light at night. The chicks were fed a standard commercial feed starter upto 14 days, thereafter a grower diet upto 28 days and finisher upto 35 days. Chickens were allowed access to the diets and fresh and clean drinking water *ad libitum*. All the experimental chicks were kept under close observation during entire period of study.

Experimental design

Individually weighed chicks were randomly divided into 4 groups of 30 chicks; each group consisting of 3 replicates of 10 chicks. Chicks of group-I was kept as untreated control and was given only basal diet. Chicks of groups II, III and IV were given diet with selenium @ 2, 6 and 10 ppm respectively from day first of experiment for 35 days. Six broilers from each group were used for assessing the cell mediated immunity. The remaining broilers from each group were kept for haematological, biochemical and pathological study. When the chicks reached 35 days of age, the feeding trial was terminated.

Cell mediated immunity (CMI)

Cell mediated immune response was measured by Di Nitro Fluro Benzene (DNFB) test as described by Phanuphak *et al.*, (1974) and later slightly modified by Tamang *et al.*,

(1988). Featherless area was marked on both sides of abdomen and cleaned thoroughly with acetone and air dried. Right lateral side of abdomen was used for DNFB application whereas left side served as control. 2000µg of DNFB in 0.1 ml of acetone and olive oil (4:1) was applied on the right marked area on the abdomen using a plastic ring to avoid spillage. The sensitized birds were challenged with 50µg of DNFB in 0.1 ml of acetone and olive oil (4:1) on the same area on 14th day after initial sensitization. The response to DNFB was assessed by measuring the skin thickness using engineer's micrometer on 0, 24 and 48 hours of post challenge with three readings each and the overall mean skin thickness was calculated.

Haematology

At the end of the experiment on day 35 blood samples were collected in heparinised vials from jugular veins. Thin blood smears were prepared for differential leukocyte count during blood collection. The total leucocytic count (TLC) was done as per Jain (1986), by using W.B.C. diluting fluid (Merck Limited, Mumbai - 400018) and Haemocytometer (Neubauer's chamber and WBC diluting pipette).

The differential leucocytic count (DLC) was done as per Coles (1986), using Leishman's stain (Merck Limited, Mumbai- 400018). The percentages of different leucocytes were determined by examining the stained blood smear under oil immersion objective lens of light microscope.

Histopathology

The tissue samples of spleen were collected in 10% neutral buffered formalin for histopathological studies. The tissues were thoroughly washed in running water; dehydrated in ascending grades of alcohol;

cleared in benzene and embedded in paraffin at 58°C. The paraffin embedded tissue sections of 4 to 5 µm were obtained and stained with haematoxylin and eosin (H and E) as per the method described by Bancroft and Stevens (1990) with slight modifications. The stained sections were examined under light microscope and the lesions were recorded.

Statistical analysis

Data obtained in different parameters were statistically analyzed by using complete randomized design (CRD)-single factor analysis of variance by Snedecor and Cochran (1968).

Results and Discussion

Cell mediated immune response (CMI)

In the present study the mean increment in abdominal skin thickness of treated broilers at different hours post challenge were depicted in Table 1. Broilers exposed to the challenge dose of DNFB exhibited erythema, oedematous changes and vesicle and scab formation.

Broilers of all Se treated groups had pronounced changes than that of control. Present study indicated that the contact sensitivity to DNFB could be conveniently applied in broilers for studying CMI response using abdominal skin in place of comb as test site unlike the previous method (Tiwari and Goel, 1984).

Control broilers had significant decrease in abdominal skin thickness as compared to selenium treated broilers at 24 and 48 hours post challenge which clearly indicated the immunosuppression due to selenium toxicity. Present findings of significant decrease CMI response on DNFB test was in agreement with

the findings of earlier workers in alphamethrin intoxicated broilers (Singh *et al.*, 1999).

Contact hypersensitivity is a T- cell mediated cutaneous immune response to reactive haptens (Elmets and Bowen, 1986). After exposure of the skin to contact allergens, haptens covalently bind to discrete amino acid residues on carrier proteins.

The epidermal Langerhans cell, a member of the dendritic-cell family, takes up haptenated proteins and processes them into antigenic peptides which are transported to the cell surface in association with major histocompatibility complex molecules (Wang *et al.*, 2001).

Matos *et al.*, (2005) reported that DNFB induces the activation of the extra cellular signal-regulated kinases ERK1/2 and p38, and also up regulates CD40 expression.

Haematology

Results on the haematological alteration due to subacute selenium toxicity in broilers were given in Table 2. A significant ($p \leq 0.05$, 0.01) decrease in leucocyte count was observed in broilers of all intoxicated groups. The present study also showed that selenium caused a significant eosinopaenia and leukopaenia due to lymphopaenia in all the intoxicated broilers. This finding was in agreement with the changes induced by Se in broiler chickens earlier (Kumar *et al.*, 2011). Marked leucopaenia in subacute selenium toxicity in the present study was probably due to their cumulative effect following daily administration. Continuous exposure to Se may then lead to lymphopaenia, which may have an immunosuppressive effect in broilers. The marked lymphopaenia in the present study might have occurred due to the toxic effect of sodium selenite on bone marrow and stress (Goyal *et al.*, 1986).

Table.1 DNFB response (mean increase in skin thickness in mm) of broilers exposed to subacute selenium toxicity (Left side served as vehicle control and right side treated with DNFB)

Groups	Abdominal side	Before sensitization	After sensitization	
			24 hr	48 hr
Gr I	Left	0.55±0.04	0.63±0.08	0.61±0.08
	Right	0.58±0.04 ^a	3.22±0.10 ^b	3.47±0.16 ^b
Gr II	Left	0.57±0.05	1.81±0.17	1.77±0.22
	Right	0.61±0.05 ^a	3.37±0.09 ^b	3.64±0.09 ^b
Gr III	Left	1.25±0.07	1.68±0.09	1.65±0.09
	Right	1.33±0.08 ^b	1.90±0.14 ^a	2.71±0.18 ^a
Gr IV	Left	1.15±0.05	1.47±0.16	1.47±0.17
	Right	1.21±0.06 ^b	1.65±0.13 ^a	2.86±0.13 ^a
Level of significance		**	**	**

Table.2 Effect of induced subacute toxicity of Selenium on haematological changes in broilers

PARAMETERS	GROUPS				Level of significance
	Group I	Group II	Group III	Group IV	
TLC (10^3 /cu.mm)	31.18±0.36 ^d	29.19±0.21 ^c	27.21±0.15 ^b	26.08±0.27 ^a	**
Lymphocyte (%)	74.5±1.12 ^b	64.5±2.19 ^a	61.83±1.42 ^a	60.83±0.60 ^a	**
Heterophil (%)	16±0.93 ^a	19.5±1.45 ^b	20.16±1.11 ^b	21.83±0.48 ^b	**
Monocyte (%)	5.33±0.80 ^a	13.33±0.67 ^b	14.5±0.62 ^b	14.66±0.49 ^b	**
Eosinophil (%)	2.83±0.31 ^b	0.83±0.31 ^a	1.33±0.21 ^a	0.83±0.31 ^a	**
Basophil (%)	1.33±0.21 ^a	1.83±0.17 ^a	2±0.16 ^a	1.83±0.17 ^a	NS

Values indicate Mean ± S.E. Superscripts may read row wise for comparison of means. NS - No significance difference. (*P≤0.05) and (**P≤0.01)

Fig.1 Section of spleen (group III) showing oedematous changes. H & E × 400

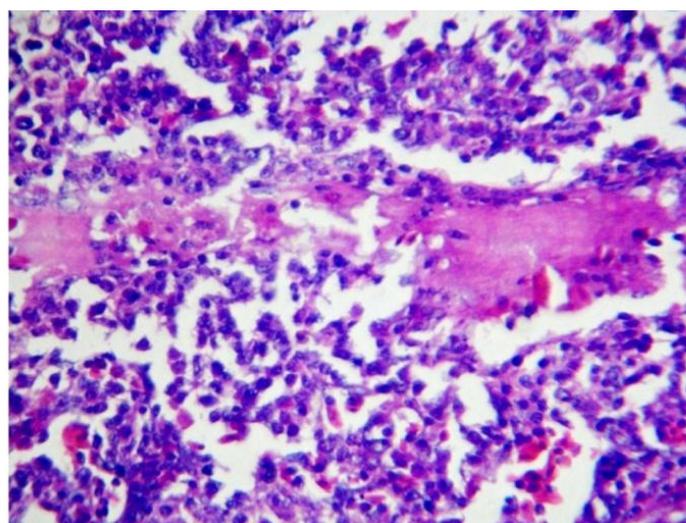


Fig.2 Section of spleen (group IV) showing severe depletion of lymphocytes and vacuolation.
H & E \times 400

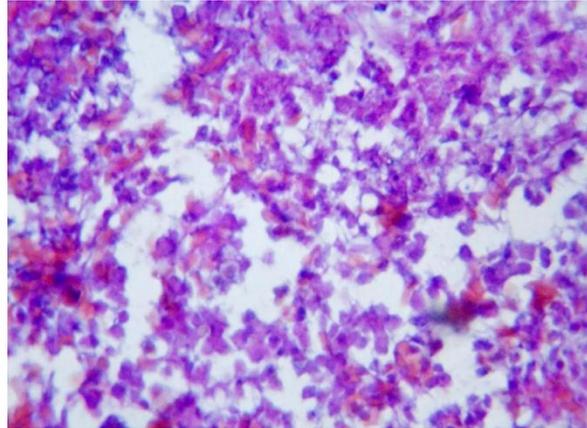


Fig.3 Section of bursa of Fabricius (group IV) showing vacuolation and severe depletion of lymphocytes. H & E \times 400

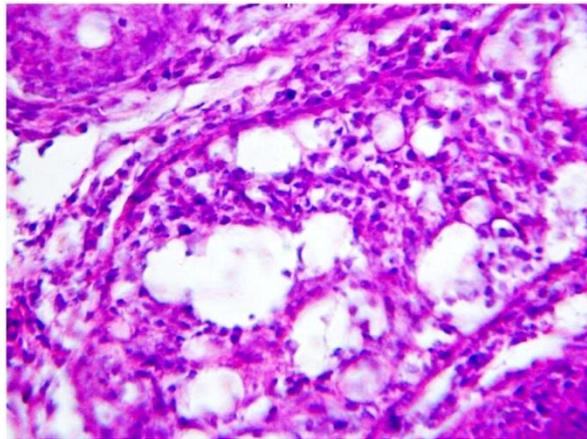
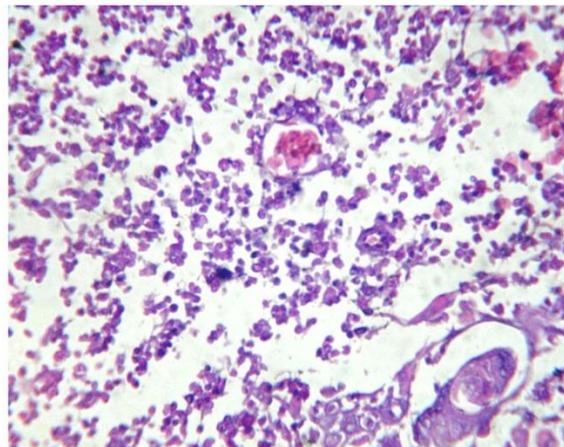


Fig.4 Section of thymus (group IV) showing severe medullary lymphocytic depletion.
H & E \times 100



Histopathology

Histologically the spleen of intoxicated broilers had congestion, oedematous changes (Fig. 1), severe depletion of the lymphocytes and vacuolation (Fig. 2). The histopathological changes of spleen were closely corroborated with the findings of Jacevic *et al.*, (2011). In the present study the marked lymphocytolysis of the germinal center of the splenic follicles correlates with the findings related to suppression of the CMI and indicated that selenium causes immunosuppression. Microscopically induced broilers revealed serous exudation, vacuolation and depletion of lymphocytes in the follicles of bursa of Fabricius (Fig. 3). Thymus of Se intoxicated broilers had congestion, haemorrhages, oedematous changes and severe medullary lymphocytic depletion (Fig. 4). Histological changes of lymphoid organs in selenium induced broilers were in close conformity with the findings of Narayani (2010) who reported severe lymphocytosis in the germinal center of the spleen, bursa of Fabricius and thymus in alphamethrin treated broilers. So, the present study suggested that the selenium toxicity causes immunosuppression in the broilers.

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