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Immune system modulation and cytological changes in response to ethion intoxication in poultry birds

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Abstract

Present study was done to delineate the immune-regulated interplay following the toxicological deterring effect of ethion. The study was conducted in chicks and they were equally and randomly divided into four groups (n=6) with group I served as control, group II, III and IV were administered with ethion at 2.5, 5.0 and 7.5 mg/kg body weight per os route respectively for 30 days. On 30th day the blood and tissue samples were collected to assess the immunotoxic potential of ethion. The study revealed significant ($P < 0.05$) reduction in total protein, albumin and globulin, splenic cell cellularity count, lymphocytic count whereas significant ($P < 0.05$) increase was observed in dose dependent manner on apoptotic cell count via FACS. The non significant changes were observed in HI titre, DTH response and lymphocyte proliferation assay following 30 days administration of toxicant to chicks. Histopathological examination of spleen and skin tissue revealed more pronounced alteration in the normalcy of spleen and skin in group III and IV as compared to control. The study therefore concluded that ethion intoxication in poultry birds produced significant ($P < 0.05$) apoptosis in splenocytes, heterophilia and lymphocytopenia as a part of defensive mechanism albeit at given experimental doses direct immunotoxic effect was not advocated. Further downstream apoptotic signaling studies are warranted.

Key Words: Apoptosis, Chicks, Ethion, Immunotoxicity, Pesticide

Introduction

Pesticides play a pivotal role in the growing economy of the developing countries as they provide the thrust to improve the feed and food production by counter- playing with the invading pests and insects. With the advent of new agrochemicals and shift of the focus towards regulatory ethics on use of these chemicals, the incidence of accidental toxicity cases have been reduced. Pesticides can be a potential source of contamination to water bodies such as ponds, lakes etc. and also to the soil and plants. The particulate matters in the environment and leaching of these substances in the Pedological strata might result in delayed toxicosis. India is one of the largest

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importers of pesticides in the world. Among these insecticides, ethion a member of organophosphate class imposes a great threat to human and animals. Chemical name of ethion is O,O,O',O'-Tetraethyl S,S'-methylene bisphosphorodithioate. It is colorless to light brown or pale yellow colored compound with liquid state at room temperature. It is having mild dithiophosphate like odor with a boiling point of 164-165°C at 0.3 mm Hg and specific gravity ranges from 1.215-1.230 at 20°C[24].

The mass ethion toxicity episode of Ahmadabad Gujarat revealed the toxicological potential of ethion[5]. Organophosphate poisoning is quite common and fatal[1]. The immune system of human as well as animals is of prime sentinel to the pesticide and other xenobiotics. Pesticide exposure may result into immune competence or may result into immune stimulation that might be the cause of variety of immunological disorders and development of other ailments including carcinogenesis[4]. A correlation does exist between the disrupted development of neuronal tissue and disturbed demeanor and psychology of organism[9]. Ethion is a small (MW 384), lipid-soluble molecule that can be absorbed by passive diffusion through the lungs, gastrointestinal tract, or skin. Absorption appears to be rapid by the oral and dermal routes; the time course of absorption is inferred from the onset of clinical signs within 1 hour after accidental ingestion of ethion in a 6-month-old boy[3] and deaths within 3–6 hours (hrs) in dermally exposed Sherman rats[6].

Global exposure of these pesticides induces the immunological insult in the animals and birds. Major mode of production of immunosuppression is production of reactive oxygen species, mitochondrial dysfunctioning and reduction of CD15 cells generation and thereby autoantibody production[18]. Ethion is commonly used against bushes and hedges of kitchen gardens and backyards. Chicken are reared as backyard farming in India and abroad and the exposure to this compound may altered the immunological status and thus conferred the disturbed neuro-physiological behavior and functions. *Neoschongastia americana* (Hirst), chigger lives in the soil and induces the dermatological ailments in turkey and other poultry birds. Ethion was found to be effective against these chiggers[15]. These chemicals act by interfering with the activities of cholinesterase, an enzyme that is essential for the proper working of the nervous systems of both humans and insects. Further, ethion was considered as one of the toxic pesticide to birds and reduces the plasma cholinesterase level by around 30 %[16]. Inhibition of cholinesterase by ethion occurs in a dose dependent manner[21]. Ethion is an organophosphate pesticide used to kill aphids, mites, scales, thrips, leafhoppers, maggots and foliar feeding larvae. It may be used on a wide variety of food, fibre and ornamental crops, including greenhouse crops, lawns and turf. Ethion is often used on citrus and apples[15,26]. It is available in dust, emulsifiable concentrate, emulsifiable solution, granular and wettable powder formulations. Products containing ethion must bear the signal word "Warning"[12]. United States Environment Protection Agency (USEPA) has established re-entry intervals of from 2 to 30 days, depending on the crop, for ethion[24].

Although the inadvertent alteration in immune system by several insecticides has been documented[9] albeit the data for ethion toxicity with specific reference to immunological insult is lacking *vis* a *vis* the molecular mechanism of ethion toxicity has not been yet understood. Ethion induced inflammatory changes and effect on immune system in other species viz. mice[25] is studied but data regarding impact of ethion on poultry birds' immune system is missing. The present study was conducted to abridge the void between the underlying mechanism of development of immunotoxicity and its effect on the immune system of birds.

Materials and Methods

1. Chemicals

Ethion, of technical grade (>90% purity) used in the study, was procured from, M/s, Indofil Industries Ltd. Mumbai, India. The desired concentrations of this toxicant were prepared in corn oil. All other chemicals and reagents used in

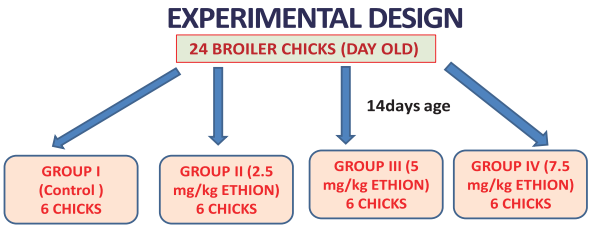

Figure 1. Experimental design of in vivo 30 days toxicity trial of ethion in chicks (n=6).

the study were obtained from Hi media, sigma and thermo scientific and the kits were obtained from ERBA diagnostics, Mumbai, India. Fluorescein isothiocyanate annexin-V- propidium iodide (FITC annexin-V-PI) apoptosis detection kit with propidium iodide and cell staining buffer were obtained from Biolegend Inc., (San Diego, CA, USA).

2. Experimental animals and Experimental design

24 day old chicks were selected for this study at Indian Veterinary Research Institute, Deemed University, India. The birds were acclimatized for 14 days before the start of experiment. They were provided with fresh water and feed, each day, *ad libitum*. Temperature and relative humidity was maintained at 37-38 degree centigrade and 60-65 per cent till 3rd week and after that was maintained to 21-22 degree centigrade and 55-60 per cent, respectively till the end of the experiment. Natural or less than 12 hours light hours was maintained throughout the experiment. Proper hygiene was maintained during the experimentation. At 2 weeks of age, 24 chicks were divided randomly and equally into four groups of 6 chicks in each. Group I was kept as control whereas, groups II, III, and IV were provided with ethion at 2.5, 5.0 and 7.5 mg/kg body weight orally, respectively, through *per os* route for a period of 30 days (Figure 1). At the end of the experimental period, birds from each group were sacrificed by humanely and blood and spleen samples were collected for estimation of following parameters. All the experimental protocols were done after the ethical approval from the Institutional Animal ethics committee of IVRI, Izatnagar, Bareilly.

3. Biochemical examination

Total protein, albumin, globulin and Albumin: globulin ratio was estimated using standard protocol mentioned in the kits[8, 10].

4. Differential leukocyte count (DLC)

DLC was done by preparing thin blood smear from a drop of blood without anticoagulant. The smear was air dried and stained with Leishman's stain for 20 minutes. The leucocytes were counted by zig-zag method and recorded on % basis.

5. Evaluation of ethion toxicity on Immune system

5.1 Effect on Humoral Immune (HI) response

5.1.1 Broilers Immunization

For evaluation of HA titre, Broilers were immunized by intra nasal injection of NCD vaccine in saline on 14th day of experiment. Booster dose was given at the interval of 14th day again by the same route.

5.1.2 Titration of haemagglutinating serum antibodies

Haemagglutination inhibition test was performed as per the standard protocol to calculate the titre. Briefly, 0.025 ml of Phosphate buffer saline (PBS) was dispensed into each well of a plastic V-bottomed microtitre plate. 0.025 ml of serum was placed into the first well of the plate. Two fold dilutions of 0.025 ml volumes of the serum were made across the plate.4 HAU virus/ antigen in 0.025 ml is added to each well and the plate was left for a minimum of 30 minutes at room temperature, i.e. about 20°C, or 60 minutes at 4°C. 0.025 ml of 1% (v/v) chicken Red Blood cells (RBCs) is added to each well and, after gentle mixing, the RBCs were allowed to settle for about 40 minutes at room temperature, i.e. about 20°C, or for about 60 minutes at 4°C if ambient temperatures are high, when control RBCs settled to a distinct button. Hemagglutinin Inhibition (HI) titre was the highest dilution of serum causing complete inhibition of 4 HAU of antigen. The agglutination is assessed by tilting the plates. Only those wells in which the RBCs stream at the same rate as the control wells (positive serum, virus/

antigen and PBS controls) were considered to show inhibition. The validity of results was assessed against a negative control serum, which should not give a titre >1/4 (>22 or >log2 2 when expressed as the reciprocal), and a positive control serum for which the titre should be within one dilution of the known titre.

5.2 Evaluation of Cell Mediated Immune Response

5.2.1 Evaluation of delayed type hypersensitivity (DTH) response

DTH response was estimated by using the 2,4 Dinitro Chloro Benzene (DNCB) dye test for monitoring the cell mediated immunity. It was done before 48 hours of slaughtering the experimental birds after primary sensitization at day 14th of study. Skin over left and right lateral abdomen of each bird was defeathered, aseptically cleaned and 2"×2" area was marked with India ink. Primary sensitization was carried out by intra dermal injection of 0.1 ml of 0.5% DNCB dye in acetone on day 14th on left side, while on right, acetone 0.1 ml was injected. Skin thickness was measured with vernier calipers at 0, 12, 24, and 48 hours after challenge. The sensitized birds were challenged after 14 days i.e. on 30th day with 0.1 ml of 0.05% DNCB solution in acetone on left side lateral abdominal skin, while right side was injected with acetone only.

5.2.2 Assessment of reaction

Reaction was assessed by measuring the skin thickness at 0, 12, 24 and 48 hours after challenge with the help of vernier caliper on 12, 24 and 48 hours after the challenge. Histopathology of abdominal skin was also performed to study cellular changes in DTH reaction.

5.3 Lymphocyte proliferation assay in vitro

Lymphocyte proliferation assay was performed by the method of Roehm *et al.* (1991)[17] with some modifications. A brief methodology of the protocol is described as follows:

Two mitogens were used to measure proliferation of splenic lymphocytes. T cell proliferation response was studied with concanavalin-A (con-A), while B lymphocyte proliferation response was studied with lipopolysaccharide (LPS) from E-coli 055:B55 Single spleen cell suspensions without red blood cells were prepared as noted above. After last wash, cells were re-suspended in Dulbecco's Modified Eagle Medium (DMEM) containing sterile 10% fetal bovine serum (FBS), 1% antibiotics & antimycotic, i.e. complete DMEM. Cell viability was determined by 0.4% trypan blue dye exclusion test. Finally, the viable cell number was adjusted to 2 x 10^6cells/ml with complete medium. Test was performed in flat bottom 96 wells tissue culture plates. Triplicate cultures of each sample were made with and without mitogen. Lymphocyte cell suspension was added as100 µl/ well and then 100 µl of 5 µg/ml of con-A and 100 µl of 2.5 µg/ml of LPS was added in each of the triplicate cell suspension. To the wells containing no mitogen (cell control), 100 µl of medium was added. Corner rows and columns were filled with 200 µl of medium which apart from serving medium control, helped in tackling evaporation of well contents. The plate was covered with lid and transferred to humidified CO_2 incubator at 37 degree centigrade, 5 % CO_2 for 72 hours. After 72 h of incubation, number of proliferating cells was determined with 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide (MTT) dye. 20 µl of MTT (5 mg/ml in DMEM, thoroughly vortexed and filtered with the help of 0.22 µm syringe filter) was added to each 200 µl of culture. Plates were gently shaken to mix the contents. After additional 3-4 hours of incubation at 37°C in humidified CO_2 incubator, plate was removed and approximately 210 µl of medium was discarded from each well. Formazan crystals formed were dissolved by adding 100 µl of Dimethyl sulfoxide (DMSO) to each well and absorbance of wells was determined using an enzyme-linked immunosorbent assay (ELISA) microplate reader at wavelength of 570 nm. Before taking reading, plates were shaken for 60 seconds on plate shaker so as to dissolve crystals uniformly. Stimulation index (S. I.) was calculated as absorbance of the sample stimulated by con-A/LPS divided by absorbance of the sample without stimulation by mitogen.

5.3.1 Estimation of cell death through apoptotic / necrotic pathway using FITC annexin V staining:

The splenic cell cellularity count was done in the single cell suspension of spleen. The apoptotic and necrotic cell count was conducted using flow cytometry through Annexin V and Propidium iodide (PI) dye methods. Positioning of quadrants on Annexin-V/PI dot plots was performed and living cells (Annexin V/PI), early apoptotic/primary apoptotic cells (Annexin V+/PI), late apoptotic/secondary apoptotic cells (Annexin V+/PI+) and necrotic cells (Annexin V/PI+) were distinguished. Therefore, the total apoptotic proportion included percentage of cells with fluorescence Annexin V+/PI and Annexin V+/PI+. For Annexin-V/PI analysis, splenocytes (after last wash) were resuspended in 200 µl of Annexin V binding buffer at a concentration of 2 x 10^6 cells/ml. An aliquot of 100 µl of each sample was incubated with 5 µl Annexin-V– Fluorescein isothiocyanate (FITC) and 10 µl of PI for 15 min in dark at room temperature followed by addition of 400 µl Annexin V binding buffer. The FITC and PI fluorescence was measured immediately through FL-1 filter (530 nm) and FL-2 filter (585 nm) respectively, and 10,000 events were acquired. At least three independent experiments were performed. The flow cytometric analysis was done on a Becton Dickinson flow cytometer. Cell debris, characterized by a low forward scatter (FSC)/ side scatter (SSC) was excluded from analysis. The data was analyzed by Cell Quest software and mean fluorescence intensity was obtained by histogram statistics.

6. Histopathological examination

Skin tissues and spleen tissue samples of 1*1 cm^2 were collected and fixed in 10% Neutral Buffer formalin (NBF) for 48 hrs and subjected to further histopathological processing. Overnight washing of samples was done in running tap water. The samples were then dehydrated in ascending grades of alcohol (50%, 70%, 90%, 95%, absolute alcohol I, and absolute alcohol II for 30 minutes each). Clearing of tissues was done in two changes xylene (for 30 min each). Samples were then impregnated with paraffin for 2 hrs at 58°C. Paraffin blocks of tissue were then prepared using L molds. Tissue sections of 5-micron thickness were cut using the LEICA RM2245 Microtome and the slides were prepared. The prepared slides were then routinely stained with hematoxylin and eosin as per the conventional method[11] and mounted with DPX. The microphotographs were obtained at 40X for ease of analyzing the histoarchitectural changes.

7. Statistical analysis

Results are expressed as Mean ± S.E. with an equal the number of broilers per group. The significance was analyzed by using Microsoft Excel and SPSS statistics- 20' software by employing one-way analysis of variance (ANOVA) with Tukey's multiple comparison post hoc tests. Comparisons among treated and untreated groups were made with the help of student's' Test. Statistically, significant difference was considered at 5 and 1% level i.e. ($P < 0.05$ and $P < 0.01$) by the method of Snedecor and Cochran (1989)[23].

Results

The results of the present study revealed the significant delineation of the involvement of immune system in the toxico-pathological attributes of ethion in poultry birds. The clinical observation suggested that there was severity in the neurological signs such as depression, fits and dullness involving staggering gait and apathy in dose dependent manner. Reduction in the globulin and lymphocyte count is consistent with the severe histopathological changes in the spleen evident from paucity of the cells in the birds of 7.5 mg/kg ethion treated group i.e. Group IV. Group IV of the experimental birds revealed most significant toxic signs as compared to other groups i.e. group II and III.

Effect of ethion toxicity on immunologically related biochemical attributes

The biochemical studies revealed significant ($P < 0.05$) decrease in total protein value and globulin value in ethion treated groups as

Table 1. Effect on total protein, albumin, globulin and Albumin: Globulin (A:G Ratio) following daily oral administration of ethion for 30 days in chicks (Mean ± S.E., n = 6).

Parameters/Groups	Group I	Group II	Group III	Group IV
Total protein (g/dl)	2.989±0.0691^{C}	2.787±0.074^{B}	2.711±0.067^{B}	2.193±0.021^{A}
Albumin (g/dl)	1.591±.0593^{B}	1.516±.0536^{B}	1.541±0.078^{B}	1.245±0.0473^{A}
Globulin (g/dl)	1.397±0.032^{C}	1.271±0.025^{BC}	1.169±0.093^{B}	0.948±0.059^{A}
A:G Ratio	1.140±0.0494	1.192±0.027	1.360±0.185	1.335±0.117

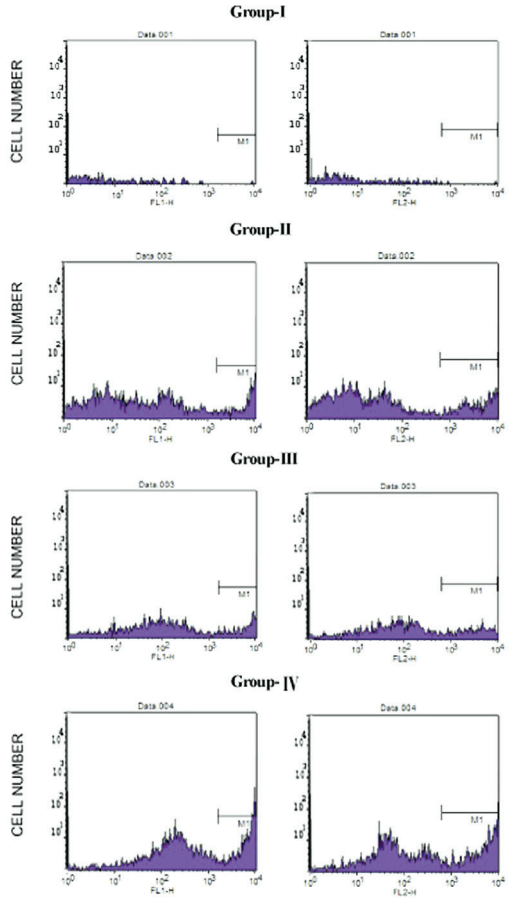

Figure 2. Effect on percentage apoptotic cells following daily oral administration of ethion for 30 days in broiler chicks (Mean ± S.E., n=6) showing dose dependent significant increase in apoptotic cell count following FITC- Annexin V staining.

compared to control group I in a dose dependent manner. However, albumin values showed a significant difference ($P < 0.05$) only in group IV as compared to group I. The perusal of data revealed a non significant change in Albumin: Globulin (A: G) ratio in all the groups (Table 1).

Effect of ethion toxicity on DLC, humoral and cell mediated immune response

The present study showed significant ($P < 0.05$) reduction in lymphocyte count in groups III and IV as compared to control group I, whereas a significant increase was observed in heterophils count in group III and IV as compared to group I. The birds of group II did not show any apparent change at 2.5 mg/kg dose level (Table 2). Further the study was cemented following immunotoxicity trial of ethion in poultry birds on both the arms of immunity. The haemagglutination inhibition titre revealed no significant change in the HI titre value (measured as log (2)/0.05 ml) in ethion treated groups II, III and IV as compared to control group I following 30 days exposure of ethion. The data of splenic cell cellularity count showed significant ($P < 0.05$) decrease in the cell cellularity in ethion treated groups as compared to control group (Table 3).

Table 2. Effect on differential leukocyte count (heterophils and lymphocytes) following daily oral administration of ethion for 30 days in chicks (Mean ± S.E., n = 6).

Groups/Parameters	Heterophils	Lymphocytes
Group I	32.00±0.816^{A}	66.25±1.250^{B}
Group II	36.62±4.830^{AB}	63.00±4.509^{B}
Group III	44.00±2.449^{B}	51.5±2.753^{A}
Group IV	41.75±0.853^{B}	56.75±3.978^{AB}

The study to observe the effect of ethion intoxication on cell mediated immunity was recorded following DTH response and lymphocyte proliferation assay. The results of DTH response study revealed a non-significant alterations in terms of skin thickness in ethion treated groups II, III and IV as compared to control after 12, 24 and 48 hrs of interval. However, maximum DTH response was generated after 24 hrs in all groups (Table 4). Lymphocyte proliferation assay following the con-A induced stimulation of lymphocytes revealed no significant change in terms of stimulation index of T cell blastogenesis, in ethion treated groups as compared to control group I (Table 4).

Table 3. Effect on HI titre and splenic cell cellularity count following daily oral administration of ethion for 30 days in chicks (Mean ± S.E., n = 6).

Parameters/Groups	Group I	Group II	Group III	Group IV
HI titre	2^7 (1:128)	2^9 (1:512)	2^7 (1:128)	2^7 (1:128)
Splenic cell cellularity	8.082 ± 0.099^{D}	6.722 ± 0.091^{B}	7.360 ± 0.086^{C}	5.797 ± 0.076^{A}

Table 4. Effect on DTH response, per cent apoptotic cells and T cell blastogenic activity following daily oral administration of ethion for 30 days in chicks (Mean ± S.E., n = 6).

	DTH Response (Skin Thickness in mm)			
Time Interval/Group	**Group I**	**Group II**	**Group III**	**Group IV**
0 hrs	2.275±0.0184	2.207±0.026	2.210±0.033	2.237±0.121
12 hrs	3.852±0.061	3.737±0.070	3.812±0.051	3.805±0.075
24 hrs	4.192±0.033	4.072±0.061	4.130±0.058	4.092±0.055
48 hrs	3.832±0.030	3.840±0.043	3.742±0.038	3.820±0.045
	% Apoptotic cells following Annexin V –PI study by FACS			
% apoptotic cells	3.04 ± 0.34^{D}	27.98 ± 0.956^{C}	30.29 ± 1.23^{B}	43.32 ± 0.856^{A}
	T cell blastogenic activity in terms of OD at 550 nm **(Stimulation Index)**			
LST value at 550 nm	0.241±0.008	0.246±0.006	0.260±0.011	0.255±0.007

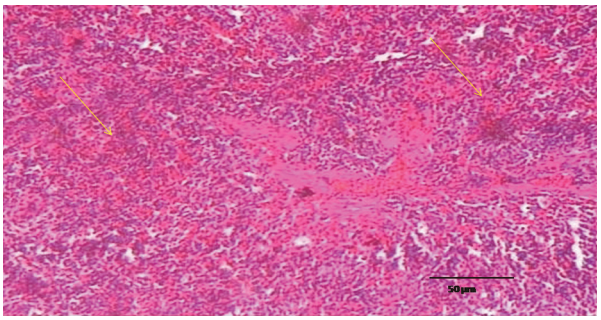

Figure 3. Microphotograph of spleen showing congestion and oedema in group II (H&E 400x)

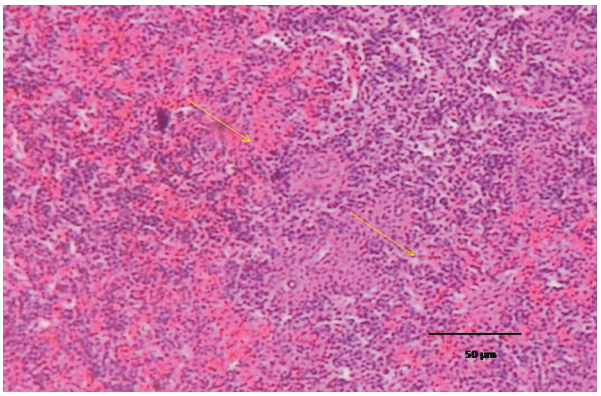

Figure 4. Microphotograph of spleen showing congestion, hemorrhages and edema in group III (H&E 400x)

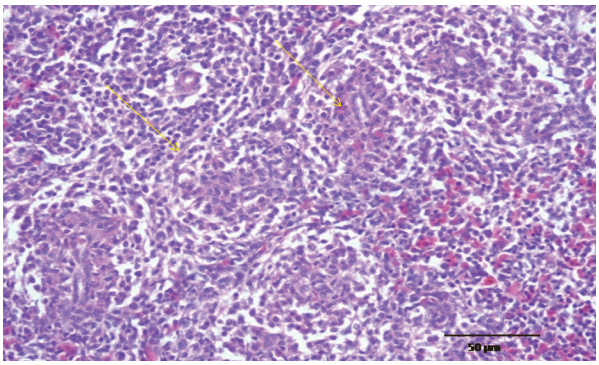

Figure 5. Microphotograph of spleen showing congestion and Rarefaction with paucity in group IV (H&E 400x)

Effect of ethion toxicity on apoptosis following FITC- Annexin V staining

The present study was aimed at evaluating the effect of ethion on apoptosis following FITC-Annexin V staining. The perusal of data advocated a significant ($P < 0.05$) increase in percent apoptotic cells in ethion treated groups i.e. II, III and IV as compared to control group in a dose dependent manner (Table 4 and Figure 2).

Effect of ethion toxicity on histoarchitecture of spleen and skin tissues

The histopathological findings of spleen revealed moderate to severe depletion of lymphocytes with mild to severe congestion in ethion treated group III and IV. The group IV tissue revealed necrosis and paucity of cells with severe hemorrhages. Rarefaction of lymphoid cells was also noticed in group III and IV. Group II revealed almost non significant histoarchitectural defect but edema was noticed (Figure 3–5). The histological examination of skin of left abdomen collected after 48 hrs, revealed varying degree

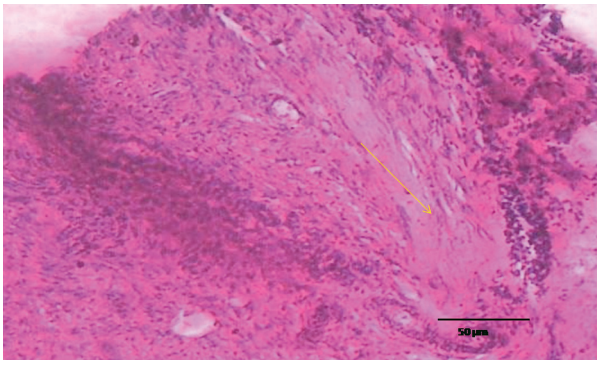

Figure 6. Microphotograph of skin showing DTH reaction oedema in group III (H&E 400 X)

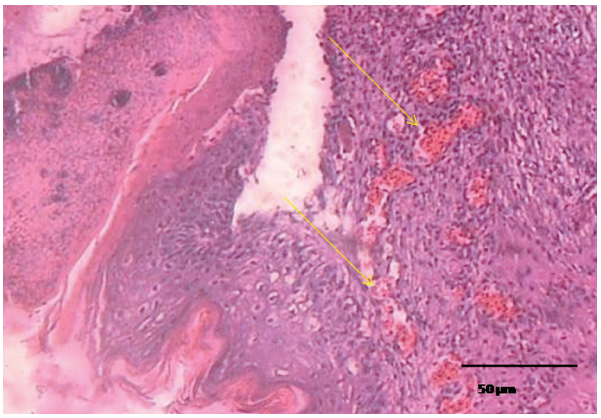

Figure 7. Microphotograph of skin showing DTH reaction, congestion oedema and cellular infiltration in group IV (H&E 400x)

of inflammatory reactions including vascular congestion, hemorrhage and cellular infiltration in control group I. The histological reactions of inflammation were almost of similar severity in all intoxicated group as compared to control group I (Figure 6, 7).

Discussion

The perusal of present data revealed significant ($P < 0.05$) reduction in total protein, globulin and albumin values in intoxicated groups. These findings were in agreement with Sankar *et al*. (2012)[19] who observed similar results following exposure of agrochemical i.e. cypermethrin in rats. The reduction in the protein, albumin and globulin could be attributed to renal excretion or impaired protein synthesis or due to liver disorders. The change in the protein profile may be related with the dysfunction of hepato-renal system and gastroenteritis. The study conducted by Shukla *et al*. (2019)[21] also revealed the hepatic insult following ethion intoxication in poultry birds. Thus dysfunction of hepatic health might be the contributory factor for reduction in the above values. The decrease in serum total protein also may be attributed to loss of protein either by decrease protein synthesis or increased proteolysis or degradation[19]. Reduction in the protein value contributed to lowered muscle mass in the broiler birds and poor Feed conversion ratio thus hampering the cost of rearing of the birds.

The reduction in lymphocyte count and increase in heterophils count is indicative of acute injury to body tissue and damage to immune related organs and tissues such as spleen etc. Observed heterophilia and lymphopenia might have occurred due to adverse effect of pesticide on normal functioning of bone marrow and stress[9]. The level of serum antibodies against New castle disease (NCD) virus antigen is the conventional index of humoral immunity[7]. The present study revealed a non significant change in HI titre in all groups as compared to control except group II. The study thus suggested that ethion treatment at 2.5 mg per kg could stimulate the humoral arm of immunity. The perusal of experimental data obtained following cell mediated immunity also revealed no significant change in skin thickness after 12, 24 and 48 hrs of DNCB challenge. Further, the lymphocyte stimulation test revealed the non significant change in T cell blastogenesis in ethion treated groups as compared to control group. Morrow (1985)[14] also suggested that cutaneous exposure of ethion in rats would not cause significant change in immune functioning. Poor immunological status confers reduction in the antibody productions and makes the birds more prone towards the infections of the naturally harboring pathogens viz. Salmonella and other enterobacteriacae members.

The apoptotic cells were examined following FITC/ Annexin V method showed significant increase in apoptotic cells in ethion treated group in dose dependent manner. The apoptosis process is triggered by several factors such as exposure to

pesticides[2]. Ethion, an organophosphate pesticide thus could ignite the intracellular machinery to stimulate apoptotic pathways. Further, the data of splenic cell cellularity count and DNA fragmentation assay suggested the apoptosis or programmed cell death in the splenic tissue in the present study as evident by significant decrease in cell count following 30 days oral exposure to ethion in poultry birds. Ethion induced apoptosis through oxidative damage as indicated by significant increase in ROS generation measured by DCFH-DA dye in flow cytometry analysis in splenocytes[21].

Histopathological study revealed necrosis and paucity of cells with severe hemorrhages in spleen of birds of group III and IV. The study was in a positive correlation with Singh and Sharma (2002)[22] who noticed necrotic changes along with the rarefaction of lymphoid cells of spleen with bytachlor toxicity in poultry. The damage depicted in splenic histoarchitecture might be suggestive of oxidative stress induced apoptosis. Study revealed that exposure of ethion in poultry birds produced significant apoptosis in splenocytes and heterophilia and lymphocytopenia had also been encountered as a part of defensive mechanism against pesticide exposure. The changes revealed a significant impact on the cost economics of the poultry farming. Study also advocated the legitimate use of these pesticides in the agriculture and household practices to mitigate the challenges of accidental poisoning to human and animals inclusive of birds. It is also concluded that ethion in the given doses did not produce significant change in immunological parameters and thus do not confer immunotoxicity potential at given doses for a given period of time. Further studies to explore the apoptotic pathways and downstream signaling pathways are warranted and strict policy papers should be reframed to counter the threat of pesticide accidental poisoning.

Conflict of Interest

The authors declare no conflict of interest.

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