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Immunosuppressive effect of combined citrinin and endosulfan toxicity in pregnant Wistar rats

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ABSTRACT

In the present investigation, citrinin (CIT) (10 mg/kg feed) and endosulfan (1mg/kg bw) were administered orally alone and in combination to pregnant Wistar rats from gestational day 6 to 20 to study their effect on the immune response of the pregnant Wistar rats. Cell mediated Immune response was assessed by delayed type hypersensitivity against ovalbumin, and lymphocyte transformation test (LTT) using MTT (3-(4,5-dimethyl thiazol-2-yl) 2, 5-diphenyl tetrazolium bromide). The humoral immune response was judged by Hemagglutination against SRBCs and indirect ELISA. Cell mediated immune response in citrinin (Gr I) was more compared to endosulfan (Gr II) treated rats as at 72 hrs the ear pinna thickness in citrinin was more than endosulfan (0.76 ± 0.011 mm vs 0.68 ± 0.006 mm), and identical findings were seen in stimulation index by LTT (1.00 ± 0.001 vs 0.90 ± 0.001). In humoral immune response the trend changed, by hemagglutination the animals in group I (citrinin) showed less titre than group II (endosulfan) (2.83 ± 0.31 vs 4.00 ± 0.26), however, by indirect ELISA the titre of citrinin (gr I) was more than that of endosulfan (gr II) (0.081 ± 0.003 vs 0.073 ± 0.001). However, in the combined group the animals were significantly more immunocompromised when adjudged for cell mediated (DTH: 0.57 ± 0.008 mm; LTT: 0.80 ± 0.007) and humoral immune responses (HA: 2.33 ± 0.21; indirect ELISA: 0.066 ± 0.001) as compared to the individually intoxicated groups and control (DTH: 0.94 ±0.012 mm; LTT: 1.10 ± 0.001), (HA: 5.50 ± 0.22; indirect ELISA: 0.108 ± 0.006). It may be concluded that with simultaneous exposure CIT and endosulfan, are potent immunosuppressants, but when they were given together their effect was enhanced possibly due to their additional interaction in pregnant Wistar rats.

Key words: citrinin, endosulfan, humoral immune response, cell mediated immune response

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Introduction

It has been estimated that around 25% of the world's cereals are contaminated with known mycotoxins (DEVEGOWDA et al., 1998). Among the mycotoxins, aflatoxin B_1 and ochratoxin A have been given due attention, but the toxic effect of other mycotoxins such as citrinin have not been studied in spite of their adverse effects on reproduction and severe pathological effects on the kidneys and other vital organs. Ingestion of mycotoxin(s) during pregnancy may lead to various developmental defects (WANGIKAR et al., 2004).

Endosulfan, an organochlorine compound of the cyclodiene group, is primarily used as an insecticide, as well as an acaricide. Chemically, endosulfan is 6,7,8,9,10,10-hexachloro-1,5,5,6,9,9-hexahydro-6,9,methano-2,4,3,-benzodioxathiepin-3-oxide. A report from the Centre for Science and the Environment (CSE) in 2003 reported alarmingly high levels (0.055-3.875 ppm) of endosulfan in blood, fat, milk, vegetables, cashew leaves, soil and water from the Padre village of the Kasargod district of Kerala, India (CSE, 2001). Moreover, human milk samples were also found to contain endosulfan residues (SANGHI et al., 2003). Though some information is available on the toxicity of citrinin and endosulfan individually in poultry (CAMPBELL et al., 1981; KHURANA et al., 1998), reports on their toxic effects are scarce in animals. Moreover, there appears to be no report on the toxicity of the combined effect of citrinin and endosulfan in pregnant rats, although both may occur as co-contaminants under field conditions in certain areas.

Mycotoxins, particularly aflatoxin B_1 and ochratoxin A are known to be a potent agent. However, the immunotoxicity of citrinin has not been properly studied and the reports are inconclusive, contradictory and inconsistent (CAMPBELL et al., 1981; DESOUZA et al., 1999). Although both organophosphate compounds such as malathion and mycotoxins (aflatoxin B_1) have been reported to cause immunotoxicity in rats and poultry (SODHI et al., 1996), no study has been conducted to evaluate the immunotoxic effect of the interaction of citrinin and endosulfan. Immune dysfunction leading to immunosuppression can affect the survival of the host.

The present study was therefore designed to study the effect of citrinin and endosulfan alone and in combination on humoral and cell mediated immune responses of pregnant Wistar rats when exposed during gestation days 6-20.

Materials and methods

Production and analysis of citrinin. Citrinin was produced by culturing *Penicillium citrinum* (NRRL-5907), procured from the National Center for Agricultural Utilization Research (NCAUR), Peoria, Illinois, USA, on sterile maize and groundnut (JACKSON and CIEGLAR, 1978) (1:1). Thin layer chromatography (TLC) and spectrophotometry methods were used for the quantitative determination of the toxin (SINGH et al., 2006) against the standard citrinin procured from Sigma Chemicals Ltd, USA.

Experimental animals. The present study was carried out using sexually mature (180 ± 10 g) Wistar female rats, procured from the Laboratory Animal Resource (LAR) Section of the Institute. All animals were house-caged in a temperature controlled and artificially illuminated room (12 h light/dark cycles) free from any source of chemical contamination. All the experimental procedures were conducted as per the guidelines of the Institute Animal Ethics Committee (IAEC) and Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). After an acclimatization period of 1 week, females were mated with mature males of the same strain. The day on which a vaginal plug was found or spermatozoa were observed in the vaginal smears was designated as day 0 of pregnancy. After mating, the female rats were individually housed in polypropylene cages. Pregnant females were weighed and randomly distributed into four groups of ten animals each and treated as follows: group I, citrinin (10 mg kg^{-1} feed); group II, endosulfan (1 mg kg^{-1} body weight) dissolved in olive oil by oral intubation; group III, citrinin (10 mg kg^{-1} feed) plus endosulfan (1 mg kg^{-1} body weight); group IV, control receiving the basal feed tested negative for CIT and olive oil (0.1 ml 100 g^{-1} b.w.) orally. The dose selection criteria for the citrinin was based on the 1/20th oral lethal (LD_{50} i.e. 50 mg kg^{-1} body weight) dose of the citrinin in rats, keeping in view the body weight and daily feed consumption. The dose of endosulfan (1 mg kg^{-1} body weight) employed in the present study was lower than the dose established by the World Health Organization (WHO) for reproductive toxicity assessment in rats (World Health Organization, 1984). The suggested NOEL (No-Observed-Adverse-Effect Level) dose for endosulfan in rats is 1.5 mg kg^{-1} body weight. Since the animals used in the present experiment were pregnant, the dose was reduced to 1.0 mg kg^{-1} body weight in view of pregnancy stress. The toxins were given from day 6 to 20 of gestation to prevent preimplantation and early post-implantation losses during days 0 to 5 of gestation.

Preparation of toxicated feed. Cultured substrate containing a known amount of citrinin was added to the basal ration (tested negative for mycotoxin contamination) in such a proportion that the concentration of citrinin in the diet was 10 mg kg^{-1} feed. Aliquots were taken from the mixed diet and the toxin was quantified by thin layer chromatography and spectrophotometry to ensure the proper mixing of the toxin. The toxicated feed was freshly prepared daily and given to the pregnant rats from gestation day 6-20.

Feeding of endosulfan. Technical grade (>99.98% pure crystalline form) endosulfan procured from Shriram Chemicals Ltd, India, was dissolved in olive oil (vehicle) and orally intubated to the pregnant rats at a rate of 1 mg kg^{-1} body weight daily from days 6 to 20 of pregnancy. The treatment volume was 0.1 ml 100 g^{-1} body weight. A fresh solution of endosulfan was prepared on each day of treatment. The control animals received an equal volume of olive oil similar to those treated with endosulfan.

Immunological studies

Humoral immune response (hemagglutination test). Four rats from each group were sensitized with sheep red blood cells (SRBCs) by intraperitoneal injection of 0.25 ml of SRBCs suspended in Phosphate Buffer Saline (PBS) at the dose rate of 1.25×10^6 cells per animal (antigen) on day 6 of gestation followed by a booster dose of the antigen after 7 days. Serum was collected on day 20 of gestation. Hemagglutination (HA) was carried out by microtitration techniques according to the standard procedure (BEARD, 1980). Briefly, the HA test was performed in a U shaped micropersplex plate. Two-fold serial dilution of serum was prepared in PBS keeping the final volume of 0.05 mL in each well, except in the control well, which contained PBS alone. Then 0.05 ml of 0.5% SRBC suspension was added to all the wells. A known negative control was also included. The plate was swirled gently for mixing and uniform distribution of erythrocytes and left at room temperature for 40-45 minutes. The HA pattern (a diffused sheet of agglutinating RBCs covering the bottom of the wells) was read with the aid of a reading lens and the titre was recorded as reciprocal of the highest dilution showing complete agglutination of erythrocyte and expressed as $\log_2$/0.05 mL.

Humoral immune response (indirect ELISA). On days 6 and 12 of gestation, four pregnant dams were immunized with ovalbumin (200 µg/rat) in Freund's complete adjuvant (FCA), intraperitoneally. The rats continued to be fed on their respective rations and doses. On day 15, these rats were challenged with only ovalbumin without FCA 10 µg/rat. The blood was collected on the day 20 of gestation prior to sacrifice to obtain serum. For humoral immune response, indirect enzyme linked immunosorbant assay (ELISA) was performed (MISHRA et al., 1985). Briefly, each well of 96 well flat microtitre plates was precoated with 100 µL of ovalbumin (12.5 µg/mL) and left overnight at 4 °C. The plate was then blocked with 200 µL of 5 per cent skimmed milk powder in phosphate buffer saline containing 0.5 per cent Tween - 20 (PBST). 100 µL of pre-diluted rat serum (1:500) was added to each well and incubated at room temperature for 2 hours. 100 µL of (1:20,000) horse radish peroxidase (HRPO) conjugated goat anti - rat IgG (National Institute of Immunology, New Delhi) was added to each well and incubated at room temperature for 2 hours. Then 100 µL of HRPO substrate (1 mg/mL) ortho phenylene diamine (OPD) was added to each well and incubated in the dark for 30 minutes. The enzyme substrate reaction was stopped at 30 min. by adding 100 µL of 2N sulphuric acid. The absorbency at 492 nm for each well was read on an ELISA reader. Washing of the plates with PBST was done at each step starting from antigen coating until the addition of substrate.

Cell mediated immune response (Delayed type hypersensitivity (DTH) test). TH test was performed in different toxin fed and control group pregnant dams (four each). This was performed on day 15 of primary immunization, which was done on GD 2. Each rat

was injected with 10 µg ovalbumin in 20 µL PBS intradermally in the right ear pinna. The left ear pinna was injected with 20 µL PBS alone. The thickness of the ear pinna was measured at 0, 24, 48 and 72 h by an electronic Vernier's caliper (Forbes) and an increase in thickness of the pinna at different intervals was recorded. At each interval, the skin pieces from the ear pinnae were surgically removed after proper anesthesia and preserved in 10 per cent formalin for histopathological examination.

Lymphocyte proliferation assay. Lymphocyte proliferation test using MTT (3-(4, 5-dimethyl thiazol-2-yl) 2, 5-diphenyl tetrazolium bromide) was performed (BOUNOUS et al., 1992) on day 20th of gestation.

Isolation of peripheral blood mononuclear cells. Blood samples (3-4 mL) were drawn aseptically from heart into sterile syringes containing filter sterilized (Seitz filter) ethylene diamine tetra acetic acid (EDTA) (0.5 µg/mL) as anticoagulant, and collected in sterile tubes. The tubes were centrifuged at 1500 - 2000 rpm for 30 minutes. The supernatant (plasma) was removed. Buffy coat was collected and resuspended in 3 mL of PBS. This was then layered carefully over 2 mL of histopaque (1.077 g/mL, Sigma) and the tubes were centrifuged at 2000 rpm for 45 minutes. Leucocytes at the histopaque - PBS interface were carefully aspirated and washed three times with PBS. RBC contamination, if any, was removed by the distilled water lysis method. The resulting cells were suspended in a sufficient volume of phenol red free RPMI-GM to give the required viable cell concentration for carrying out further steps.

The cells were adjusted to give 5×10^6 cells/mL in phenol red free RPMI-GM. 100 µL of the cell suspension was added to 3 sets of triplicate wells of a RPMI-GM. The second and third sets received 100 µL of RPMI-GM containing 100 µg/mL of Con-A. The plates were incubated at 37 °C in a humidified chamber at 5% CO_2 tension for 96 h. Then 100 µL of supernatant was removed from the wells and 10 µL of MTT solution was added to all the wells. The plate was incubated further for 4 h at 37 °C. The MTT formazon was extracted from the cells using dimethyl-sulphoxide (100 µL/well). OD was taken using an ELISA reader at a test wave length of 492 nm and a reference wavelength of 650 nm. The stimulation index was calculated by using the following formula.

$$\text{Stimulation Index} = \frac{\text{OD stimulated}}{\text{OD unstimulated}}$$

Results

Humoral immune response

Hemagglutination test. The humoral immune response, as assessed by measuring the hemagglutination titre against sheep blood RBCs in terms of log (2)/0.05 mL is presented

in Table 1. The mean HA titre was significantly lower in the combination group (2.33) followed by citrinin (2.83), endosulfan (4) and controls (5.5).

Table 1. The Effect on the immune-response of pregnant rats of various treatment groups (mean±SE)

	Groups			
Parameters	I	II	III	IV
Delayed type hypersensitivity (DTH) in skin[1]				
24 h	0.80 ± 0.035^{b}	0.40 ± 0.005^{c}	0.80 ± 0.008^{b}	0.90 ± 0.014^{a}
48 h	0.86 ± 0.004^{c}	0.98 ± 0.011^{b}	0.79 ± 0.007^{d}	1.03 ± 0.008^{a}
72 h	0.68 ± 0.006^{c}	0.76 ± 0.011^{b}	0.57 ± 0.008^{d}	0.94 ± 0.012^{a}
Lymphocyte transformation test (LTT) (SI)[2]				
Stimulation Index	0.90 ± 0.001^{c}	1.00 ± 0.001^{b}	0.80 ± 0.007^{d}	1.10 ± 0.001^{a}
Hemagglutination test (HA) titre[3]				
HA Titre ($\log_2$)	4.00 ± 0.26^{b}	2.83 ± 0.31^{c}	2.33 ± 0.21^{c}	5.50 ± 0.22^{a}
Indirect ELISA[4]				
OD	0.073 ± 0.001^{c}	0.081 ± 0.003^{b}	0.066 ± 0.001^{c}	0.108 ± 0.006^{a}

Gr. I = Endosulfan (1mg/kg b.w.); Gr. II = Citrinin (10 ppm); Gr. III = Citrinin + endosulfan (10 ppm + 1 mg/kg b.w.); Gr. IV = Control; [1]Increase in skin thickness (mm); [2]Lymphocyte transformation test (stimulation index) with Con-A; [3]Hemagglutination titre against sheep RBCs. [4]Indirect ELISA OD; Means bearing at least one common superscript do not vary significantly between group ($P<0.05$).

Indirect ELISA. Indirect ELISA was performed to assess the humoral immune response after collecting rat serum. There was a significant decline in the titre of citrinin + endosulfan group rats (0.066) compared with that of group I (CIT) (0.081), group II (endosulfan) (0.073) and group IV (control) rats (0.108) (Table 1).

Cell mediated immune response

Delayed type hypersensitivity (DTH). Cell mediated immune response was evaluated by employing delayed type hypersensitivity reaction to ovalbumin. The increases in thicknesses of skin of ear pinna at 24, 48 and 72 h after the challenge with ovalbumin in different groups were measured. There was an apparent decrease in skin thickness in the group III (C+E) fed group compared with those of group I (citrinin), group II (Endosulfan) and group IV (control) animals at all intervals (Table 1). Histopathologically, the combination treated animals showed a much smaller number of infiltrating cells at 24 h (Fig. 1) and 48 h (Fig. 2). At 72 h, the reaction practically subsided. In group I (citrinin), at 24 h, there was diffuse infiltration of polymorphs and lymphocytes into the dermis and subchondral tissue (Fig. 3). The epidermis had become hyperplastic/acanthotic at

the injection site. At 48 h, there was an almost pure population of macrophages with slight engorgement of the blood vessels (Fig. 4). At 72 hours, the number of macrophages significantly decreased (Fig. 5). In group II, i.e. the endosulfan treated rats, histopathological examination of skin biopsies collected at 24, 48 and 72 h, revealed varying degrees of acute inflammation in dermis characterized by the presence of neutrophils with edema and congestion of blood vessels at 24 h (Fig. 6). The intensity of neutrophil infiltration decreased significantly, with the appearance of macrophages at 48 h (Fig. 7). At 72 h, an inflammatory granulomatous reaction still persisted, especially in the epidermis with the appearance of fibroblasts along with macrophages (Fig. 8). The control group animals showed intense cellular reaction at all three intervals (Figs 9, 10).

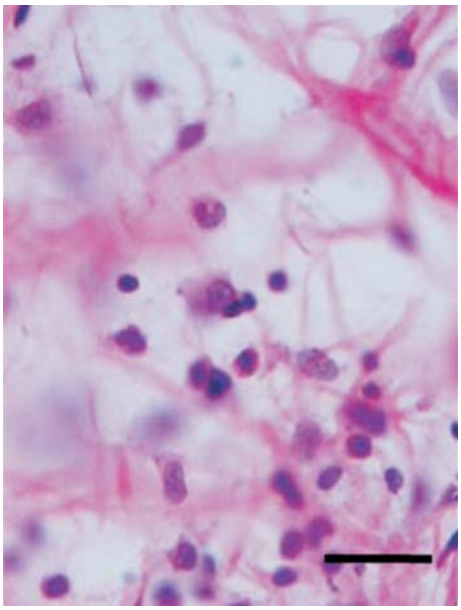

Fig. 1. (Group-III/ Combined citrinin + endosulfan) Skin DTH, 24 h: Mild cellular infiltration with macrophages and a few polymorphs in the dermis. H&E; ×400.

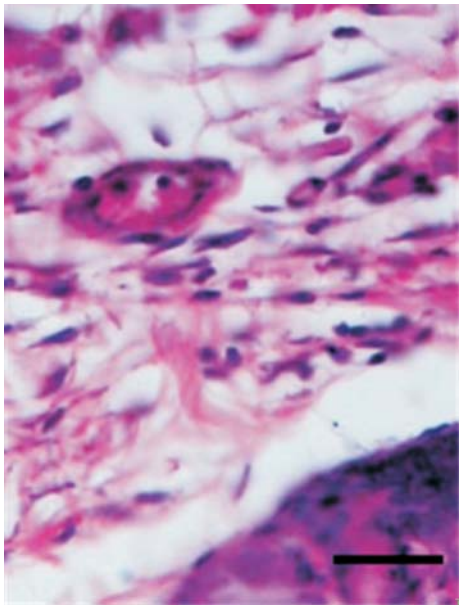

Fig. 2. (Group-III/ Combined citrinin + endosulfan) Skin DTH, 48 h: Disappearance of inflammatory cells from dermis. H&E; ×400.

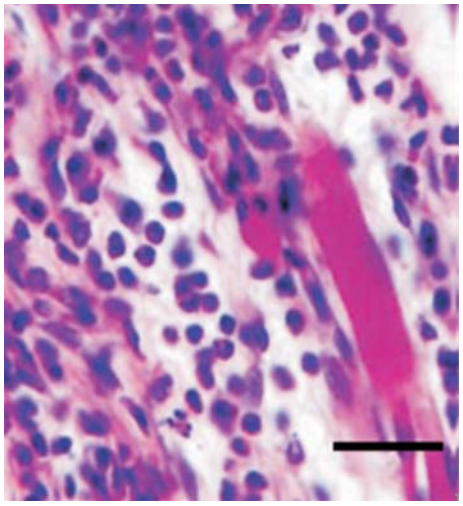

Fig. 3. (Group-I/ citrinin) Skin DTH, 24 h: Infiltration of polymorphonuclear cells alongwith few macrophages in deep dermis with oedema. H&E; ×400.

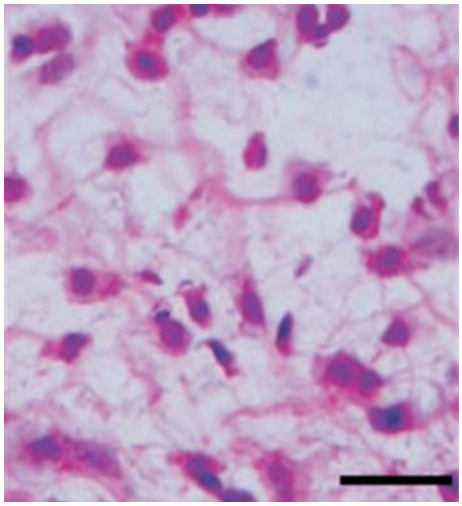

Fig. 4. (Group-I/ citrinin) Skin DTH, 48 h: Dermal oedema with sparse macrophage reaction. H&E, ×400.

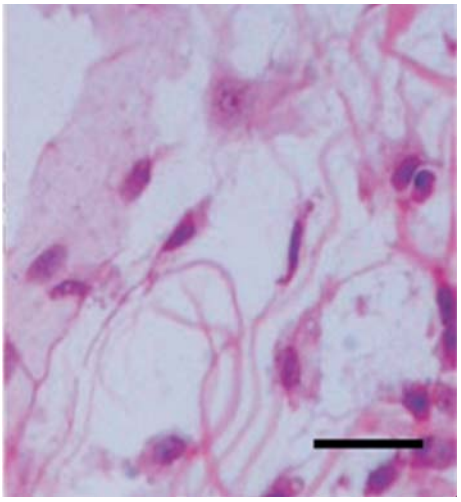

Fig. 5. (Group-I/ citrinin) Skin DTH, 72 h: Showing presence of only a few macrophages with appearance of fibroblasts. H&E; ×400.

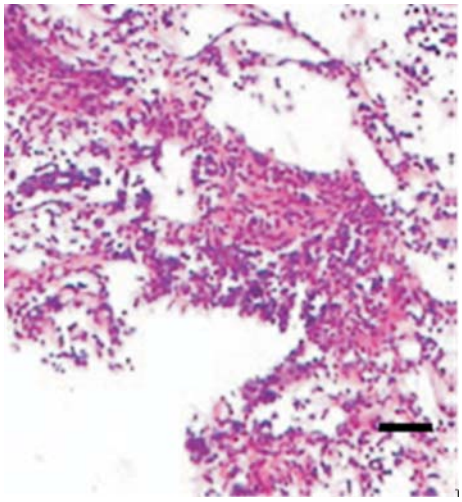

Fig. 6. (Group-II/ Endosulphan) Skin DTH, 24 h: Diffuse cellular infiltration with oedema. H&E; ×100.

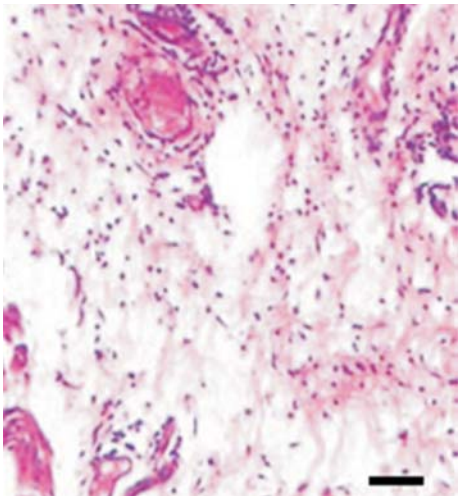

Fig. 7. (Group-II/ Endosulphan) Skin DTH, 48 h: Diffuse sparsly distributed macrophages in the dermis. H&E; ×100.

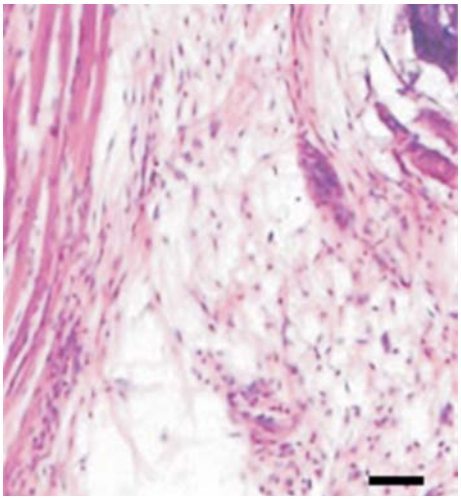

Fig. 8. (Group-II / Endosulphan) Skin, DTH, 72 h: Reduced number of macrophages and fibrin meshwork in the dermis. H&E; ×100.

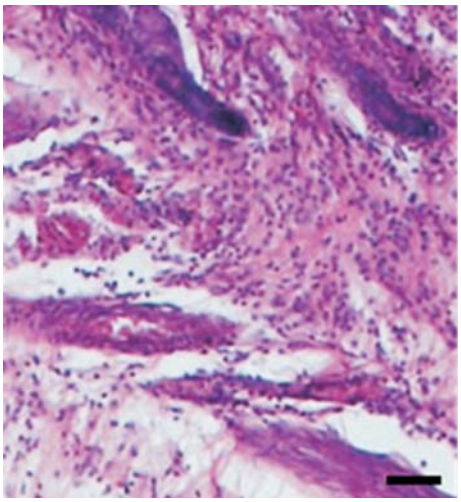

Fig. 9. (Group-IV/ Control) Skin DTH, 24 h: Intense inflammatory reaction with heavy polymorphs and MNCs infiltration with vascular congestion. H&E; ×100.

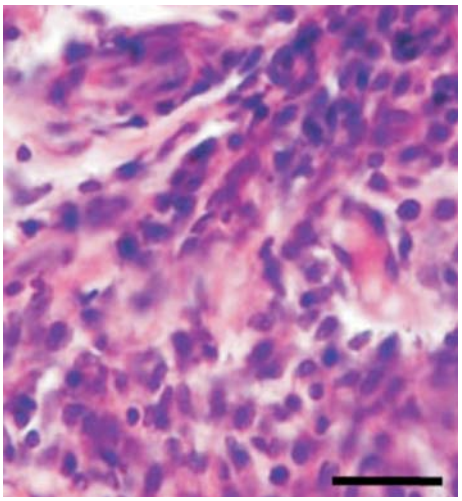

Fig. 10. (Group-IV/ Control) Skin DTH, 48 h: Heavy mononuclear cells infiltration and a few polymorphs. H&E; ×400.

Lymphocyte transformation test. LTT was performed by separating MNCs from the blood, it was sensitized with Con A and the stimulation index was measured. There was a significant difference between the SI of citrinin + endosulfan (Gr III) treated (0.8) as compared to group I, II and control (1.0 vs. 0.9 vs. 1.1, respectively) rats, it being higher in the latter case (Table 1).

Discussion

The cell mediated immunity of animals of the citrinin group also decreased significantly as assessed by DTH reaction (ovalbumin) and the lymphocyte transformation test, which could be attributed to the depletion of lymphocytes in the spleen and Peyer's patches (SINGH et al., 2007b) and also a severe degree of lymphocytopenia (SINGH et al., 2006). The findings regarding the immunotoxic effects of citrinin were inconsistent (CAMPBELL et al., 1981; REDDY et al., 1988; DESOUZA et al., 1999).

Both cell mediated (DTH, LTT) and humoral immune responses were significantly reduced in endosulfan treated rats as compared with those of the control animals. These observations found support from earlier studies in rats (BANERJEE and HUSSAIN, 1986) and poultry (KURKURE et al., 1993).

The reduction in immunoglobulin levels in the serum of rats in this group was evidenced by a significant decrease in the antibody titres in the hemagglutination test against SRBCs and Indirect ELISA for antibodies against ovalbumin, and was in accordance with earlier findings in rats (NAQVI and VAISHNAVI, 1993) and poultry (THAKER, 1988; VARSHNEYA et al., 1988a; VARSHNEYA et al., 1988b; KHURANA et al., 1998). This might be due to the immunotoxic effect of endosulfan.

The decrease in CMI in endosulfan might be attributed to the reasons already cited for the citrinin fed group. Since the lymphoid follicles (splenic corpuscles) of the spleen are responsible for B-cell production (GREAVES and FACCINI, 1984) and maturation of IgG takes place in Peyer's patches (SMINIA and PLESCH, 1982), the depression in humoral immune response could be well correlated with the depletion of lymphocytes in the spleen and Peyer's patches (SINGH et al., 2006). This was substantiated by the decreased HA titres against SRBCs and antibodies against ovalbumin assessed by indirect ELISA.

In the combination group, both CMI and humoral immune responses were markedly suppressed as compared to those of the individual treatment groups. This indicated that both citrinin and endosulfan caused immunosuppression in an additive manner. This finding is supported by an earlier study in poultry regarding a combination of aflatoxin B1 and malathion which caused marked immunosuppression (SODHI et al., 1996). However, this is the first study on the toxic effects of a combination of citrinin and endosulfan, which proves that in addition to hematobiochemical (SINGH et al., 2006), teratological

(SINGH et al., 2007a) and pathomorphological changes (SINGH et al., 2007b), these two agents also cause immunotoxicity in pregnant Wistar rats in an additive manner.

Conclusion

This is the first study on the toxic effects of a combination of citrinin and endosulfan, which proves that in addition to hematobiochemical, teratological and pathomorphological changes, these two agents also cause immunotoxicity in pregnant Wistar rats in an additive manner. So, meticulous use of pesticides and proper storage of feed is very important to protect human and animal life from the toxic chemicals which easily enter the food chain.

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References

ANONYMOUS (2001): Centre for Science and Environment (CSE), Report. 2001. Endosulfan Conspiracy. Down To Earth. New Delhi, India.

ANONYMOUS (1984): Endosulfan: International Programme on Chemical Safety. Environmental Health Criteria. World Health Organization, Geneva, 1-62.

BANERJEE, B. D., Q. Z. HUSSAIN (1986): Effect of sub-chronic endosulfan exposure on humoral and cell-mediated immune responses in albino rats. Arch. Toxicol. 59, 279-284.

BEARD, C. W. (1980): Serological procedure, isolation and identification of avian pathogens. . Creative Printing Company, NewYork. 2nd ed., pp. 129-135

BOUNOUS, D. I., R. P. CAMPAGNOLI, J. BROWN (1992): Comparison of MTT colorimetric assay and tritiated thymidine uptake to lymphocyte proliferation assays using chicken splenocytes. Avian Dis. 36, 1022-1027.

CAMPBELL, M. L., J. A. DOERR, R. D. WYATT (1981): Immune status in broiler chickens during citrinin toxicosis. Poult. Sci. 60, 1634.

DESOUZA, M. M. S., R. B. FREIRE, C. CARVALHO (1999): Evaluation of the immunomodulatory action of citrinin by stimulation of lymphocyte blastogenesis. Rev. Brasil. Med. Vet. 21, 69-71.

DEVEGOWDA, G., M. V. L. N. RAJU, N. AFZALI, H. V. L. N. SWAMY. (1998): Mycotoxin picture worldwide: Novel solution for their counteraction. In: Biotechnology in Feed Industry. (Lyons, T. P, K. A. Jacues, Eds.), Kentuky, Alltech. pp. 241-255.

GREAVES, P., J. M. FACCINI (1984). Rat Histopathology. 3rd ed. Pub: Elsevier, Amsterdam, New York, USA.

JACKSON, L. K., A. CIEGLAR (1978): Production and analysis of citrinin in corn. Appl. Environ. Microbiol. 36, 408-411.

KHURANA, S. K., R. S. CHAUHAN, S. K. MAHIPAL (1998): Immunotoxic effects of cypermethrin and endosulfan on macrophage functions of broiler chicks. Ind. J. Anim. Sci. 68, 105-106.

KURKURE, N. V., A. G. BHANDARKAR, M. V. JOSHI, R. D. SADEKAR, S. S. BHAGWAT (1993): Immunosuppressive and histotoxic effects of endosulfan in chicks. Ind. J. Anim. Sci. 63, 1258-1260.

MISHRA, S. C., A. RAI, T. N. JAISWAL (1985): An enzyme linked immunoassay for estimation of antibodies to Newcastle disease virus strains. Acta. Virol. 29, 154-157.

NAQVI, S. M., C. VAISHNAVI (1993): Bioaccumulative potential and toxicity of endosulfan insecticide to non- target animals. Comp. Biochem. Physiol. 105, 347-361.

REDDY, R. V., M. J. TAYLOR, R. P. SHARMA (1988): Evaluation of citrinin toxicity on the immune functions of mice. J. Food Protec. 51, 32-36.

SANGHI, R., M. K. K. PILLAI, T. R. JAYALAKSHMI, A. NAIR (2003): Organochlorine and organophosphorus pesticides in breast milk from Bhopal, Madhya Pradesh, India. Hum. Exp. Toxicol. 22, 73-76.

SINGH, N. D., A. K. SHARMA, P. DWIVEDI, R. D. PATIL, M. KUMAR, D. BASHEER AHAMAD (2006): Toxicity of endosulfan and citrinin alone and in combination in pregnant rats: Clinico-haematological and serum biochemical alterations. Ind. J. Vet. Pathol. 30, 26-30.

SINGH, N. D., A. K. SHARMA, P. DWIVEDI, R. D. PATIL, M. KUMAR (2007a): Citrinin and endosulfan induced teratogenic effects in Wistar rats. J. Appl. Toxicol. 27, 143-151.

SINGH, N. D., A. K. SHARMA, P. DWIVEDI, R. D. PATIL, M. KUMAR (2007b). Citrinin and endosulfan induced maternal toxicity in pregnant Wistar rats: Pathomorphological study. J. Appl. Toxicol. 27, 589-601.

SMINIA, T., E. C. PLESCH (1982): An immunohistochemical study of cells with surface and cytoplasmic immunoglobulins *in situ* in Payer's patches and lamina propria of rat small intestine. Virch. Arch. Cell. Pathol. 40, 181-189.

SODHI, S., A. P. S BRAR, G. S. GREWAL (1996): Biochemical studies on combined immunomodulatory effects of aflatoxin and malathion in broiler chicks. Ind. J. Poult Sci. 31, 88-91.

THAKER, A. M. (1988): Toxicological and immunological stuides on long term expsoure to malathion and endosulfan in WLH chicks. Ph. D. Thesis submitted to Haryana Agricultural University, Hisar.

VARSHNEYA, C., H. S. BANGA, L. D. SHARMA (1988a): Effect of insecticides on humoral immune response in cockerels. Br. Vet. J. 144, 610-612.

VARSHNEYA, C., H. S. BANGA, L. D. SHARMA (1988b): Toxicological effects of dietry endosulfan in cockerels. Ind. J. Poult. Sci. 23, 4-8.

WANGIKAR, P. B., P. DWIVEDI, N. SINHA (2004): Effect in rats of simultaneous prenatal exposure to ochratoxin A and aflatoxin B1. I. Maternal toxicity and fetal malformations. Birth Defects Res. B Dev. Reprod. Toxicol. 71, 343-351.

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SAŽETAK

Citrinin (CIT) (10 mg/kg hrane) i endosulfan (1 mg/kg tjelesne mase) bili su oralno davani skotnim Wistar štakoricama zasebno i u kombinaciji od šestoga do 20. dana skotnosti radi istraživanja njihova učinka na imunološki odgovor. Stanični imunološki odgovor određivan je na osnovi kasnoga tipa preosjetljivosti na ovalbumin i testa transformacije limfocita (LTT) upotrebom MTT (3-(4, 5-dimethyl thiazol-2-yl) 2, 5-diphenyl tetrazolium bromide). Humoralni imunološki odgovor određivan je na osnovi hemaglutinacije svinjskih crvenih krvnih stanica i neizravnoga imunoenzimnog testa. Stanični imunološki odgovor na citrinin (skupina I) bio je jači u odnosu na endosulfan (skupina II) 72 sata nakon primjene. Zadebljanje uške na citrinin bilo je jače nego na endosulfan (0,76 ± 0,011 mm vs 0,68 ± 0,006 mm), a slični rezultati bili su polučeni u testu transformacije limfocita (1,00 ± 0,001 vs 0,90 ± 0,001). Humoralni imunološki odgovor bio je drugačiji. U testu hemaglutinacije, u životinja skupine I (citrinin) ustanovljen je manji titar nego u skupine II (endosulfan) (2,83 ± 0,31 vs 4,00 ± 0,26). Međutim, neizravnim imunoenzimnim testom titar citrinina (skupina I) bio je veći nego u skupine koja je dobivala endosulfan (skupina II) (0,081 ± 0,003 vs 0,073 ± 0,001). U skupini koja je dobivala istodobno citrinin i endosulfan ustanovljen je značajno slabiji stanični imunološki odgovor (DTH: 0,57 ± 0,008 mm; LTT: 0,80 ± 0,007) i humoralni imunološki odgovor (HA: 2,33 ± 0,21; neizravna ELISA: 0,066 ± 0,001) u usporedbi sa skupinama koje su posebno dobivale citrinin odnosno endosulfan te kontrolnom skupinom (DTH: 0,94 ± 0,012 mm; LTT: 1,10 ± 0,001), (HA: 5,50 ± 0,22; neizravna ELISA: 0,108 ± 0,006). Može se zaključiti da istodobno izlaganje citrininu i endosulfanu može djelovati imunosupresivno.

Ključne riječi: citrinin, endosulfan, humoralni imunološki odgovor, stanični imunološki odgovor