Toxic manifestation of endosulfan and ochratoxin-a in adult male rats

Abstract

The study examined effects of Ochratoxin-A (OTA) and Endosulfan on the haematobiochemical changes in rats. Adult male Wistar rats were randomly divided into four groups and fed OTA @ 4ppm in feed (Group I); endosulfan @ 5mg/kg body weight in corn oil by oral intubation (Group II); combination of both endosulfan @ 5mg/kg body weight in corn oil and OTA @ 4ppm in feed (Group III) and toxin free feed (Group IV – control group) daily for 30 days. After treatment, blood was collected from heart for studying haematobiochemical indices. All toxin treated rats showed a significant (P=0.05) decrease in the body weights. The treated rats became anemic (normochromic hypochromic anemia) and also revealed lympho cytopenic-leukopenia. Biochemical changes included significant decline in, serum globulin, total protein and albumin along with concurrent increased levels of blood glucose, AST and ALT. Spleen of the treated animals showed depletion of lymphocytes on light microscopy. The present investigation revealed significant toxicity in combination group in comparison to single exposure to OTA or endosulfan alone (Group I and Group II, respectively). These findings suggest that concurrent exposure to endosulfan and OTA results in additive toxic manifestation on haematobiochemical parameters in male rats.

Keywords: ochratoxin-a (ota), endosulfan, normochromic normochromic anemia, lymphocytopenia, leukopenia

Abbreviations: OTA, ochratoxin-a; HR, hour; PPM, parts-per million; B.Wt., body weight; RBC, red blood cell; Hb, hemoglobin; PCV, packed cell volume; TLC, total leukocyte count; DLC, differential leukocyte count; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; BUN, blood urea nitrogen; ALP, alkaline phosphatase; ALT, alanine transaminase; AST, aspartate transaminase

Introduction

Contamination of agricultural produce by pesticides and/or mycotoxins can result in severe problems both for human health as well as the economic value of crops. Mycotoxins are fungal metabolites produced by several molds that contaminate various agricultural commodities during crop production, or in storage, where drying technology may be preventative. Ochratoxin-A (OTA), produced by several species of genera Aspergillus and Penicillium is a common contaminant of different food or feedstuffs which can cause toxicities in human beings and animals. OTA can result in several deleterious health effects causing – neurotoxicity, nephrotoxicity, genotoxicity, immunotoxicity, carcinogenicity and teratogenicity, in various mammalian species. Occurrence of OTA has also been reported in humans’ serum, breast milk and kidneys suggesting its public health significance.

Pesticides are used to protect crops from damage caused by pests including molds and pathogens. Exposure of the general population to pesticides can occur by direct application to food commodities (to increase their shelf-life) and/or through drinking water contaminated with pesticide residues. At low level many pesticides have potential toxic effects on non-target organisms and may interfere with the endocrine system. Endosulfan is one of the most commonly used organochlorine pesticides, which is lipophilic in nature. Because of its persistence in the environment, its usage has been banned in most developed countries. However, it is still being used in the developing countries because of its availability through illegal importation. The perused literature showed some information on the contamination OTA and endosulfan in adult male rats, but reports on their toxic effects in animals are limited. Moreover, no report could be traced in the literature on the combined effect of OTA and endosulfan in male rats, although both may occur as co-contaminants under field conditions in certain areas. It is with this view that this study was carried out to investigate the combined toxic effects of endosulfan and OTA on the hematological and biochemical indices of male rats.

Materials and methods

Production and analysis of Ochratoxin-A

A pure culture of Aspergillus ochraceus NRRL-3174 originally procured from National Centre for Agriculture Research (NCAUR-3174) Peoria, Illinois, USA was grown on sterilized maize as per the method described by Trenk et al. The extraction and clean up of the toxin sample was done as per the method of AOAC. Cultured maize powder containing known amount of OTA was added to basal ration in such a proportion that the final concentration of OTA was adjusted to 4 ppm level in the feed.

Animals and experimental design:

Male Wistar rats (n=40) with average weight 160±10g were procured from Laboratory Animal Resource Section of Indian Veterinary Research Institute (IVRI), Izatnagar, Bareilly, Uttar Pradesh, India. Animals were housed in polypropylene cages in an artificially illuminated room (12-hr light: 12-hr dark cycle) free from any source of chemical contamination. The temperature and relative humidity of the room were maintained at 22±3°C and about...
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Table 1

| Group   | Control | OTA | Endosulfan (ES) | Combination (OTA + ES) | Significance |
|---------|---------|-----|-----------------|------------------------|-------------|
| Weeks   | Body weight (gm) expressed as Mean±SE (n=10) |     |                 |                         |             |
| 0       | 155.0±8.42 | 156.67±7.26 | 156.67±6.67 | 158.25±3.12 | Non significant |
| 1st     | 157.5±7.77 | 150.0±7.64 | 156.0±3.05 | 137.5±3.23 | P≤0.05 |
| 2nd     | 160.25±7.19 | 138.67±5.20 | 155.33±2.67 | 131.5±3.01 | P≤0.05 |
| 3rd     | 165.00±6.45 | 134.33±5.36 | 151.67±3.84 | 122.5±3.23 | P≤0.05 |
| 4th     | 171.00±7.08 | 128.67±4.18 | 148.33±5.67 | 112.5±2.50 | P≤0.01 |

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Table 2 Effect of OTA and Endosulfan (ES) administered alone and in combination on hematological parameters (Values expressed as Mean±SE, N=10) in adult male rats. Mean bearing at least one common superscript do not differ significantly between groups. ES= Endosulfan

| Parameters                  | Control             | OTA (4ppm)       | Endosulfan (5 mg kg\(^{-1}\) b.wt) | Combination (OTA + ES) | Significance |
|-----------------------------|---------------------|------------------|----------------------------------|------------------------|-------------|
| Hemoglobin (g/dl)           | 13.78±0.175\(^c\)  | 9.22±0.35\(^a\)  | 12.15±0.48\(^b\)                 | 7.86±0.31\(^a\)        | P≤0.01      |
| PCV (%)                     | 40.52±0.83\(^a\)   | 37.18±0.95\(^b\) | 38.56±1.32\(^c\)                 | 31.36±1.73\(^b\)       | P≤0.01      |
| TLC (X10\(^3\)/μl)         | 12.74±44\(^b\)     | 10.42±0.54\(^a\) | 11.51±0.50\(^a\)                 | 10.13±1.11\(^a\)       | P≤0.05      |
| RBC (10\(^9\)/μl)          | 6.62±0.153\(^a\)   | 6.12±0.172\(^b\) | 6.43±0.22\(^a\)                  | 5.29±0.29\(^b\)        | P≤0.01      |
| MCV (fL)                    | 60.94±0.60\(^c\)   | 60.73±0.48\(^b\) | 60.76±0.36\(^b\)                 | 58.19±1.27\(^a\)       | P≤0.05      |
| MCHC (%)                    | 34.31±0.77\(^c\)   | 23.58±0.90\(^c\) | 31.86±1.77\(^c\)                 | 27.10±1.85\(^a\)       | P≤0.01      |
| MCH (pg)                    | 20.80±0.44\(^a\)   | 14.28±0.57\(^b\) | 19.11±0.98\(^b\)                 | 14.73±1.27\(^b\)       | P≤0.01      |
| Neutrophil (%)              | 31.30±0.70\(^a\)   | 56.0±0.10\(^c\)   | 29.5±0.90\(^a\)                  | 30.5±0.10\(^a\)        | P≤0.01      |
| Lymphocyte (%)              | 65.30±0.47\(^a\)   | 36.10±0.87\(^a\) | 65.70±0.67\(^a\)                 | 64.10±0.90\(^b\)       | P≤0.01      |
| Monocyte (%)                | 1.60±0.27\(^a\)    | 5.00±0.47\(^b\)  | 2.40±0.16\(^a\)                  | 3.70±0.47\(^a\)        | P≤0.01      |
| Eosinophil (%)              | 1.00±0.42\(^b\)    | 2.00±0.26\(^a\)  | 2.4±0.37\(^a\)                   | 1.0±0.25\(^b\)         | P≤0.01      |
| Basophils (%)               | 0.80±0.25\(^a\)    | 0.0\(^d\)        | 0.0\(^d\)                        | 0.0\(^d\)              | P≤0.01      |

Table 3 Effect of OTA and Endosulfan (ES) administered alone and in combination on biochemical parameters in adult male rats.

| Parameters                  | Control             | OTA (4ppm)       | Endosulfan (5 mg kg\(^{-1}\) b.wt) | Combination (OTA + ES) | Significance |
|-----------------------------|---------------------|------------------|----------------------------------|------------------------|-------------|
| Glucose (mg/dl)             | 80.13±2.10\(^c\)   | 100.23±2.38\(^b\)| 100.38±1.58\(^b\)               | 115.07±1.89\(^c\)      | P≤0.01      |
| Total protein (g/dl)        | 7.82±0.204\(^a\)   | 6.58±0.365\(^b\) | 7.22±0.37\(^c\)                 | 6.29±0.34\(^a\)        | P≤0.01      |
| Albumin (g/dl)              | 4.34±0.153\(^a\)   | 3.62±0.233\(^b\) | 4.05±0.223\(^a\)                | 3.08±0.244\(^a\)       | P≤0.01      |
| Globulin (g/dl)             | 3.59±0.280\(^a\)   | 2.60±0.29\(^a\)  | 2.55±0.337\(^a\)                | 2.66±0.268\(^a\)       | P≤0.05      |
| AG ratio                    | 1.84±0.075          | 1.86±0.107       | 1.78±0.17                       | 1.95±0.21              | Non significant |
| ALP (IU/L)                  | 84.60±0.194\(^a\)  | 106.7±0.62\(^b\) | 104.7±0.726\(^b\)              | 117.0±0.92\(^c\)       | P≤0.05      |
| Creatinine (mg/dl)          | 1.07±0.060\(^a\)   | 1.87±0.193\(^a\) | 1.51±0.18\(^a\)                | 2.26±0.227\(^a\)       | P≤0.01      |
| Calcium (mg/dl)             | 9.36±0.23\(^b\)    | 8.84±0.22a\(^a\) | 8.97±0.23\(^a\)                | 8.3±0.17\(^a\)         | P≤0.01      |
| Phosphorus (mg/dl)          | 8.49±0.224\(^b\)   | 8.07±0.24a\(^b\) | 8.00±0.21\(^a\)                | 7.59±0.196\(^b\)       | P≤0.01      |
| BUN (mg/dl)                 | 19.52±0.52\(^a\)   | 24.01±0.68b\(^a\)| 21.99±0.567\(^b\)              | 26.00±0.92\(^b\)       | P≤0.01      |
| ALT (U/L)                   | 79.17±3.082\(^a\)  | 103.8±2.58\(^b\) | 94.3±2.304\(^a\)               | 131.16±2.93\(^a\)      | P≤0.01      |
| AST (U/L)                   | 87.68±1.74\(^a\)   | 96.27±3.89\(^a\) | 103.18±3.76\(^a\)              | 144.45±9.28\(^b\)      | P≤0.01      |

Values expressed as Mean±SE (N=10).

Mean bearing at least one common superscript do not differ significantly between groups. ES= Endosulfan.

While samples of spleen from control animals showed the presence of parenchyma with mild hemosiderin pigment on light microscopy, sections from OTA and endosulfan treated animals showed focal depletion of lymphocytes in some of the peri-arteriolar lymphoid tissue and hemosiderin deposit in parenchyma (Figure 1 & 2). Animals exposed to Endosulfan and OTA combination had splenic corpuscles with poor population of lymphoid cells along with presence of hemosiderin pigment (Figure 3).

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Discussion

In this study, the rats of the control group as well as all the three experimental groups weighed approximately 150 to 160gm on day zero. As expected the control rats gained weight during 4-weeks experiment and their weight increased to 171±7gm. In the treatment groups, however, all the rats showed variable decline in the weight during the course of experiment and the lowest body weight was recorded in combination group treated with endosulfan and OTA. Our finding on reduction weight are consistent are with previous reports.18,19 The study further revealed OTA-induced normocytic hypochromic anemia. There was also reduction in total leukocyte count marked by lymphocytopenia along with increase in neutrophil percentage. The literature available regarding the effects of OTA on hematological parameters is contradictory and inconsistent.20,21 Mild leukocytopenia and lymphocytopenia observed in the present study could be associated with lymphoid cell depletion from lymphoid organs as observed during histopathological examination of spleen. OTA-induced leukocytopenia associated with lymphocytopenia has been reported previously in several experimental animals including rats.22 Endosulfan caused the lymphocytopenic leukocytopenia, which were reported in mice.23 Reduction in hemoglobin and PCV values, suggestive of anemia in the present study are in accordance with earlier reports in poultry,24 mice23 and pregnant rat.25 Anemia might be attributed to low nutrition intake, reduced protein synthesis and increased protein excretion in urine. Leukocytopenia was essentially lymphocytopenia type which might be correlated with decreased number of lymphocytes in spleen and Peyer’s patches seen histopathologically. Similar changes of mild depletion of lymphocytes in spleen have also been reported.26 The anemia was more pronounced in OTA and endosulfan combination group probably due to their synergistic effects on hematopoietic system.

Biochemical alterations in OTA treated rats were decrease in serum total protein, albumin, globulin and phosphorus levels. Decreased serum total protein, albumin, globulin values might be due to OTA-induced damage to liver,27 the major site of protein synthesis.26,27 OTA has been shown to inhibit the protein synthesis. Further, proteinuria especially albuminuria resulting from toxic damage to nephrons could have resulted in decreased albumin fraction observed in the study and corresponding reduction of A:G ratio.30 Decrease in calcium and phosphorus levels is in agreement with reported decline in OTA-treated Broiler chicken.31-32 Along with reduction in protein, calcium and phosphorus, OTA toxicity resulted in significant increase in the blood glucose levels. This finding was akin to the report of the earlier workers in rats33 and pig34 who recorded increase in blood glucose level in ochratoxicosis. It is possible that the depletion of glycogen from liver might have resulted in increased blood glucose levels.34 Hyperglycemia in ochratoxicosis may be due to the inhibited synthesis and/or reduced release of insulin from the pancreatic cell.33

Rats receiving endosulfan by oral intubation also showed decrease in serum total protein, albumin, globulin, calcium and phosphorous and the increase in levels of glucose, ALP, ALT, AST and BUN which is in accordance with the previous publications.35 Decrease in total protein values might be due to low feed intake, inadequate digestion and/or absorption due to endosulfan induced damage in gastro-intestinal tract, liver damage, anorexia and hepatotoxic activity of endosulfan.35 As in the case of OTA-induced toxicity, severe damage to nephrons could result in proteinuria especially albuminuria thereby causing decrease in serum albumin fraction and corresponding reduction in A:G ratio. Decrease in levels of calcium and phosphorous
has also been reported earlier. Increased blood urea nitrogen (BUN) may be due to prerenal causes (cardiac decompensation, water depletion due to decreased intake, and increased protein catabolism) or renal causes (tubular necrosis) and is in agreement with earlier reports in rats. Significantly increased blood glucose level in OTA and endosulfan treated animals is in agreement with the previous findings of increased blood glucose level after oral endosulfan and dimethoate administration and could be due to decreased level of insulin synthesis from pancreatic cells, increase in gluconeogenesis and glycogenolysis as well as inhibition of gluconeolysis and glycogenesis during stress.

Conclusion

It can be deduced from this study that individual as well as combined exposure of OTA and endosulfan has the potential to damage the structural integrity and physiological activities of the organism which led to changes in haemato-biochemical parameters. Further, concomitant exposure to more than one toxin may result in additive toxic manifestations.

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Conflict of interest

The authors declared no potential conflicts of interest with respect to the authorship and/or publication of this article.

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