Original Article

Comparative Hematoxicity of Fusirium Mycotoxin in Experimental Sprague-Dawley Rats

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ABSTRACT

Mycotoxins are fungal toxin and contaminated to human through food-stuffs. Hematological abnormality, mainly thrombocytopenia and leukopenia are induced after consumption of mycotoxin. Experiments were conducted to evaluate the hematotoxicity of trichothecenes mycotoxins in Sprague-Dawley rats. Hematological parameters viz. Hemoglobin, hematocrit, erythrocyte count (RBC), white blood cell count (WBC), lymphocytes, monocytes, neutrophils, eosinophils, basophils, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, red blood cell distribution width, mean platelet volume, plateletcrit and platelet distribution width were determined at 0, 6, 12 and 24 h after injection of 0.5 ml of T-2, Deoxynivalenol (DON), nivalenol, zearalenone, neosolaniol, ochratoxin-B mycotoxin equivalent to 1×10^{-3} $\mu g/\mu l$ to Sprague-Dawley rats. Experiments showed that trichothecenes toxin produces severe hematological alternation. The reductions of RBC and WBC were observed in all *Fusarium* mycotoxins treated group. T-2 toxin group shows severe toxicity as compared to other mycotoxin treated group. DON is the least hematotoxicity and T-2 the most.

Key words: Hematotoxicity, mycotoxins, Sprague-Dawley rats

INTRODUCTION

Fusarium fungi are widely distributed in hot and humid climatic region especially, in north-eastern parts of India. Mycotoxins are mold-produced toxins obtained from *Fusarium* fungi, which have a potential that contaminate a wide variety of foods and produces mycotoxicoses. Mycotoxicoses are characterized as feed-related, non-transferable, non-infectious diseases[1] and ingestion of mycotoxins causes a wide range of toxic responses, from acute toxicity to long-term health disorders. Human toxicosis induced by consumption of food-stuffs contaminated with mycotoxin and a common major symptom is a hematological perturbation manifesting principally as thrombocytopenia and leukopenia. The patient has rapidly progressed with coagulation problems and compromised resistance to infections and consequently, leads to septicemia and massive hemorrhages.[2] Sometimes, mycotoxins from naturally contaminated grains are more toxic than an equivalent dose of purified toxin[3] and probably due to the presence of unidentified mycotoxins and precursors in the contaminated grains, which results synergistic effects among mycotoxins.[4] Mycotoxins are apparently innocent and do not cause acute disease but later it produces toxicity and associated with multiple interacting factors that can modify the expression of toxicity.[5] This investigation presents experimental and comparative evidences of hematotoxicity of six mycotoxins viz. T-2 toxin, Deoxynivalenol (DON), nivalenol (NIV), zearalenone (ZEA), neosolaniol (NEO), and ochratoxin-B (OTB).

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MATERIALS AND METHODS

Chemicals and reagents

T-2 toxin, DON, NIV, ZEA, NEO, OTB toxin and all other chemicals were procured from Sigma-Aldrich (St Louis, MO, USA). Acetonitrile were evaporated from T-2, DON, NIV, ZEA, NEO and OTB (All toxins were supplied in acetonitrile) and dissolved in sterile water and sometimes alcohol were used for co-solvent (below 0.005%) for increasing the solubility of toxin and stored at 4°C until use.

Animals

Sprague-Dawley rats weighing 250-320 g procured from Laboratory animal resources, Division of Pharmaceutical Technology, Defence Research Laboratory, Tezpur, India and were maintained under temperature-controlled rooms at animal house with 12 h alternating light and dark cycles were given adequate nutrition and water *ad libitum*. All experimental protocols using animals were performed according to the "Principles of Laboratory Animal care" (National Institute of Health, USA publication 85-23, revised 1985) and all the protocols were approved by institutional animal ethical committee.

Experimental design

Animals were divided into seven groups and each group contained six animals. Group (I) (n = 6) were injected interperitonealy equivalent to 0.5 ml sterile water (Control group); Group (II, III, IV, V, VI, VII) (n = 6), were injected 0.5 ml of T-2, DON, NIV, ZEA, NEO, OTB equivalent to 1×10^{-3} µg/µl (treated group) through intraperitoneal route. A 500 µl blood was withdrawn from rats by tail vein puncture on hours 0, 3, 6 and 24 h after the intoxication of mycotoxin and kept in non-vacuum anti-coagulant blood collection tube (nVAC tube, HXS Tech Co. Ltd. PRC, USA). Hemoglobin, hematocrit (HCT), erythrocyte count (RBC), white blood cell count (WBC), lymphocytes (Lym), monocytes (Mon), neutrophils (Neu), eosinophils (Eos), basophils (Bas), mean corpuscular volume, mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration, red blood cell distribution width (RDW), mean platelet volume, plateletcrit and platelet distribution width were determined by automatic blood-cell counter (Milet Sesloesing Laboratory, MS-4, Osny, France) as per following the manufacturer's instructions.

Statistical analysis

The means of the groups were compared with analysis of variance using the Newman-Keul's test to correct for multiple comparisons. $P < 0.01$ was considered statistically significant.

RESULTS

The hematology results were divided into two parts including RBCs, WBCs indices and the differential counts percentage, which was mentioned in [Tables 1 and 2], respectively. The RBC, WBC, and packed cell volume shows significant reduction in T-2 toxin treated group ($P < 0.001$) and platelets showed no any significant changes. WBC counts were significantly reduced in linear fashion ($P < 0.001$) in T-2, DON and NEO treated group when comparison was done the 3, 6 and 24 h data respectively whereas the NIV and OCT-B not responded. Table 2 shows the derived factor of hematology parameter and the differential count showed no significant effect of intoxication expect for the % HCT, and MCH values. The percentage of differential counts viz. Lym, Mon, Neu, Eos, and Bas of blood were significantly ($P < 0.001$) decreased in T-2 treated as compared to other toxin treated rats, whereas DON showed minimal toxicity as compared to other toxin treated rats.

DISCUSSION

Mycotoxin is a secondary metabolite of filamentous fungi, which is low-molecular-weight and highly stable in natural conditions. Contamination of foods and feed with mycotoxins are a significant health problem worldwide, and it has been estimated that 25% of the world's crops may be contaminated with mycotoxins.[6] In the present investigation, we have evaluated the comparative hematotoxicity of six major mycotoxins viz. T-2, DON, NIV, ZEA, NEO and ochratoxin B. Presence of mycotoxins significantly declined the total WBCs, granulocytes, Lym, and platelets whereas RBC was not affected. Our findings are particularly robust because we carried out extensive ranges of hematological parameters for evaluation the comparative hematotoxicity of mycotoxins. Further, we showed that hematological abnormality was more pronounced at 24 h after intoxication as compared to 3 or 6 h. The abrupt decreasing of HCT observed within 24 h in T-2 treated group as compared to another group, which suggested that hematopoietic tissue may be affected by T-2 toxin. It is unlikely that the drop in HCT was due to hemorrhage or hemolysis: No bloody discharge was observed from any orifice, the animals remained bright and alert and the plasma and serum samples showed no evidence of hemolysis during the 24 h period when the HCT was reduced. Alteration of hematological parameters by mycotoxin particularly T-2, NIV, ZEA provides a clue that the toxin has direct or indirect actions into multiple organs. Hayes and co-worker elegantly demonstrated that the T-2 toxin was suppressing hematopoiesis in the bone marrow and splenic red pulp of the mice, which resulted HCT values were significantly reduced. Another study reported that T-2 toxin administration decreased HCT values[7]

Table 1: Showing comparative hematological changes after intoxication with different mycotoxin in Sprague-Dawley rats

Toxin	Hours	WBC (m/mm³)	Lym (%)	Mon (%)	Neu (%)	EOS (%)	BAS (%)	RBC (m/mm³)
Control	0 h	5.64±0.76	53.90±3.55	16.60±2.55	27.35±2.55	3.15±0.46	0.29±0.055	5.82±0.55
	3 h	5.90±0.52	55.10±2.43	15.98±4.09	26.22±4.05	4.90±0.44	0.20±0.085	5.09±0.32
	6 h	5.53±0.46	53.70±2.45	16.90±3.25	26.90±2.88	3.89±0.50	0.22±0.090	6.99±0.81
	24 h	5.54±0.39	54.66±3.43	17.11±3.80	27.41±2.67	4.41±0.55	0.24±0.062	5.70±0.86
T-2	0 h	5.64±0.55	52.70±1.77	16.98±3.05	27.35±2.55	3.00±0.42	0.24±0.066	5.33±0.40
	3 h	5.30±0.71	32.95±2.56**	16.57±3.33	27.30±2.54	2.22±0.42*	0.60±0.088**	4.73±0.71
	6 h	5.00±0.88	13.22±2.08**	12.60±3.41*	72.55±4.09*	3.62±0.65	0.25±0.091	4.20±0.89*
	24 h	2.29±0.49*	21.15±1.90*	16.78±3.09*	34.86±3.70**	3.24±0.55	0.20±0.070*	1.78±0.56**
DON	0 h	5.77±0.89	53.67±2.09	16.55±4.22	26.88±3.11	2.78±0.42	0.23±0.073	5.55±0.59
	3 h	1.63±0.42**	42.1±3.78*	19.77±4.33*	34.82±2.54*	3.22±0.34	0.45±0.086**	3.38±0.76*
	6 h	4.66±0.80*	15.55±2.45**	20.45±3.77*	61.15±4.20*	2.79±0.42*	0.45±0.088*	2.92±0.54**
	24 h	2.52±0.49**	38.90±4.16*	27.25±5.11*	45.55±3.23	3.96±0.55	0.15±0.069	5.15±0.33*
NIV	0 h	5.29±0.49	54.33±3.07	15.87±2.90	27.90±2.11	2.72±0.41	0.25±0.057*	4.97±0.59
	3 h	4.25±0.77	47.98±2.90	20.44±4.54*	29.50±1.98*	2.34±0.49*	0.15±0.075	3.21±0.55**
	6 h	3.06±0.70*	24.45±3.11*	12.28±3.33**	59.82±3.21**	2.90±0.52*	0.75±0.034**	2.88±0.49**
	24 h	4.81±0.66	25.16±2.50*	31.49±2.80*	39.05±3.44**	3.98±0.47*	0.55±0.080*	4.07±0.45*
ZEN	0 h	5.79±0.80	52.67±3.33	16.03±3.50	25.89±2.09	2.66±0.42	0.24±0.076	5.33±0.69
	3 h	3.88±0.44**	24.53±2.09*	8.25±0.66**	64.22±3.07**	2.73±0.53*	0.35±0.058*	3.21±0.43**
	6 h	5.10±0.62	21.12±2.90*	17.45±2.22*	58.22±2.81**	3.75±0.70*	0.75±0.059**	2.84±0.39*
	24 h	3.76±0.81*	23.24±3.60*	32.05±3.81*	40.35±3.07*	4.15±0.62**	0.40±0.022*	3.65±0.48*
NEO	0 h	5.55±0.78	54.91±4.09	16.39±2.50	26.77±2.32	2.65±0.33	0.27±0.092	5.20±0.55
	3 h	4.75±0.67*	18.25±3.95*	32.06±2.99*	54.87±1.80*	4.15±0.49*	0.50±0.065**	3.65±0.60**
	6 h	4.60±0.45	11.65±3.02**	22.30±3.77*	68.12±2.22*	2.55±0.41**	0.40±0.070*	3.10±0.39*
	24 h	1.94±0.50**	29.54±2.68	17.03±3.81**	37.85±4.47*	3.70±0.40	0.40±0.070	2.39±0.44**
OCT-B	0 h	5.47±0.75	53.40±3.80	16.55±3.87	26.50±2.44	2.82±0.51	0.29±0.033	5.33±0.69
	3 h	3.81±0.77*	35.08±2.92*	16.30±2.99	43.33±4.98*	4.66±0.52*	0.45±0.070*	2.18±0.46**
	6 h	2.9 0±0.89**	25.85±4.10*	27.87±2.22*	42.45±5.90*	3.75±0.45**	0.60±0.022**	2.30±0.55**
	24 h	6.79±0.66*	19.65±2.44**	30.20±3.59*	46.00±4.73*	3.60±0.78*	0.55±0.079**	3.54±0.60*

WBC=White blood cell count, Lym=Lymphocytes, Mon=Monocytes, NEU=neutrophils, EOS=Eosinophils, BAS=Basophils, RBC=Erythrocyte count,*= P<0.05, ** = P<0.001

and caused cellular damage to bone marrow[8,9] in cats. Another study, hematological toxicosis (thrombocytopenia and leucopenia) were observed in humans by ingestion of trichothecenes and the study showed that DON is the least myelotoxic and T-2 the most powerful toxin[10] and our study shows similar results where T-2 toxin proved as potential hematotoxic with a comparison of other mycotoxins. According to the European commission of scientific committee on food and the joint FAO/WHO expert committee on food additives (JECFA) toxicological profiles of DON, NIV, T-2 and HT-2 are similar and NIV is a potential hematotoxic.[11] In other toxicological studies, four mycotoxins T-2, HT-2, DAS and DON were evaluated on human platelet progenitors (CFU-MK) cells and results confirmed that at low concentrations of mycotoxins imparted cytotoxic effects in megakaryocyte progenitors, which may contribute thrombocytopenia.[12] ZEA also exhibits cytopathic effects on isolated human peripheral blood mononuclear cells and 30 μg/ml ZEA was found to totally inhibit T and B lymphocyte proliferation from the stimulation with phytohemagglutinin and pokeweed mitogen.[13] Richietti and another co-worker[10] reported that citrinin, ochratoxin B, rubratoxin B, and zearalenol beta–that affected superoxide anion production were studied on human neu with regard to hypochlorous acid generation, nitric oxide formation, and chemotaxis of isolated cells. In our study, after 24 h, NIV showed the dramatic loss of NEU, HCT and RBC whereas other hematological parameter was unchanged and T-2 showed the most powerful hematotoxic as compared to other mycotoxins.

Our investigation shows that presence of mycotoxins total WBC's, granulocytes, Lym, B cells and platelets and derived other hematological parameter significantly declined in different degree. T-2 toxin is potential mycotoxins as compared to other five mycotoxins DON, NIV, ZEA, NEO, OTB. Several mycotoxins are associated with and implicated in human and animal diseases and need to establish maximum levels, guidelines or action levels for them in

Table 2: Showing comparative hematological changes after intoxication with different mycotoxin in Sprague- Dawley rats

Toxin	Hours	MCV (fl)	HCT (%)	MCH (pg)	RDW (g/dl)	Hb (g/dl)	MPV (fl)	PCT (%)
Control	0 h	66.80±2.56	39.05±2.98	10.35±1.11	12.47±2.65	11.97±1.85	7.5±0.66	0.90±0.03
	3 h	64.22±4.11	38.98±3.06	10.88±2.41	12.44±2.22	11.55±1.25	7.2±0.58	0.87±0.08
	6 h	65.54±4.56	37.96±2.58	9.87±0.98	11.59±1.45	12.36±2.00	6.9±0.45	0.89±0.06
	24 h	64.47±3.90	38.55±3.44	11.32±2.14	12.03±2.07	11.58±2.17	7.1±0.82	0.93±0.07
T-2	0 h	65.88±3.52	39.74±2.78*	9.55±0.87*	12.44±2.41	12.46±2.15	7.21±0.66	0.90±0.05
	3 h	47.75±3.89*	38.22±2.56**	23.45±2.11	9.45±0.87**	11.23±1.54*	6.05±0.91*	0.11±0.04*
	6 h	49.60±4.50*	20.85±2.11*	20.8±3.45*	10.42±0.91*	8.80±0.99**	6.44±0.69	0.13±0.02**
	24 h	48.05±2.87**	13.77±2.14**	22.25±4.02*	9.95±0.73**	6.45±0.78**	5.20±0.45**	0.08±0.06
DON	0 h	66.33±3.26	39.00±3.89*	10.11±1.24**	11.50±1.12*	11.33±2.54*	7.72±0.87	0.82±0.05
	3 h	47.75±2.66*	19.86±3.22**	22.10±2.78*	9.58±0.87*	9.45±0.22**	5.30±0.36**	0.33±0.09**
	6 h	45.65±3.28**	11.90±2.01**	27.15±2.56	10.27±0.88	9.00±0.42*	5.70±0.78*	0.15±0.07*
	24 h	48.20±4.23	14.13±3.33**	19.10±2.45**	8.25±0.58**	5.64±0.74**	5.10±0.55*	0.15±0.08
NIV	0 h	66.00±3.03	38.22±3.66*	9.55±0.88**	11.87±1.45*	12.07±1.33*	6.93±0.87	0.87±0.05
	3 h	46.50±2.56*	25.52±2.54*	19.66±1.45*	9.25±0.72*	10.15±0.54	4.95±0.21*	0.26±0.02*
	6 h	47.55±3.66**	27.12±2.87*	23.45±1.21*	10.35±0.73	7.24±0.55**	6.01±0.33	0.16±0.04*
	24 h	47.40±2.98**	19.10±2.54**	20.97±2.11*	9.40±0.88**	6.33±0.89**	5.80±0.54*	0.16±0.05**
ZEN	0 h	63.22±5.22	39.05±2.55*	10.66±2.45	12.00±1.24	11.87±1.25	7.33±0.74	0.97±0.07
	3 h	49.25±3.69**	19.75±3.47**	22.88±2.33*	10.35±0.87**	9.43±0.66*	5.25±0.45**	0.20±0.04**
	6 h	46.30±4.21*	14.95±3.40**	21.47±2.78*	9.07±0.65*	7.06±0.85**	5.60±0.79*	0.19±0.02*
	24 h	47.20±4.44*	13.35±2.65**	22.15±2.55*	9.45±0.87*	6.34±0.45**	5.60±0.62*	0.12±0.05**
NEO	0 h	65.81±6.30	38.55±5.41	9.98±0.87	12.65±1.45	12.87±1.22	7.02±0.87	0.96±0.08
	3 h	50.00±5.21*	18.44±3.11*	22.05±2.12**	11.35±1.09*	7.85±0.47*	6.80±0.54*	0.07±0.009**
	6 h	47.30±4.91*	14.57±2.10*	20.79±3.56**	9.42±0.54**	6.44±0.89*	5.05±0.91**	0.11±0.05*
	24 h	47.00±3.28*	11.22±2.54**	23.26±2.84*	9.15±0.58**	5.67±0.52*	5.40±0.88**	0.10±0.04*
OCT-B	0 h	66.89±3.55	38.55±3.44	11.42±1.25	12.44±1.33	12.33±1.33	8.98±0.74	0.88±0.05
	3 h	47.75±2.98**	24.95±2.87*	20.11±2.58*	10.08±0.87*	5.35±0.58**	5.35±0.70*	0.18±0.06*
	6 h	48.20±2.36	13.95±3.41**	21.25±2.44*	10.25±0.98*	5.80±0.45*	5.80±0.54*	0.15±0.06*
	24 h	47.50±2.45*	16.80±2.45**	19.85±1.87**	9.35±0.80**	5.70±0.77*	7.00±0.39	0.13±0.05*

Values expressed as mean±SD, *n*=6. Significantly different (*P<0.01, P<0.001) from control group. MCV=Mean corpuscular volume, MCH=Mean corpuscular haemoglobin, RDW=Red blood cell distribution width, HCT=Hematocrit, Hb=Hemoglobin, MPV=Mean platelet volume, PCT=Plateletcrit, DON=Deoxynivalenol, NIV=Nivalenol, ZEN=Zearalenone, NEO=Neosolaniol, OCT-B=Ochratoxin-B, ** = P<0.001

some kinds of commodities. Therefore, the regulatory guideline should be imposed to take administrative actions for elaboration of legislation and implementing regulatory measures for the control of mycotoxins contamination.

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REFERENCES

1. Bennett JW, Klich M. Mycotoxins. Clin Microbiol Rev 2003;16:497-516.
2. Parent-Massin D. Haematotoxicity of trichothecenes. Toxicol Lett 2004;153:75-81.
3. Harvey RB, Kubena LF, Huff WE, Elissalde MH, Phillips TD. Hematologic and immunologic toxicity of deoxynivalenol (DON)-contaminated diets to growing chickens. Bull Environ Contam Toxicol 1991;46:410-6.
4. Smith TK, McMillan EG, Castillo JB. Effect of feeding blends of *Fusarium* mycotoxin-contaminated grains containing deoxynivalenol and fusaric acid on growth and feed consumption of immature swine. J Anim Sci 1997;75:2184-91.
5. Morgavi DP, Riley RT. An historical overview of field disease outbreaks known or suspected to be caused by consumption of feed contaminated with *Fusarium* toxins. Anim Feed Sci Technol 2007;137:201-12.
6. Fink-Gremmels J. Mycotoxins: Their implications for human and animal health. Vet Q 1999;21:115-20.
7. Hayes MA, Bellamy JE, Schiefer HB. Subacute toxicity of dietary T-2 toxin in mice: Morphological and hematological effects. Can J Comp Med 1980;44:203-18.
8. Lutsky I, Mor N, Yagen B, Joffe AZ. The role of T-2 toxin in experimental alimentary toxic aleukia: A toxicity study in cats. Toxicol Appl Pharmacol 1978;43:111-24.

9. Sato N, Ueno Y, Enomoto M. Toxicological approaches to the toxic metabolites of Fusaria. VIII. Acute and subacute toxicities of T-2 toxin in cats. Jpn J Pharmacol 1975;25:263-70.
10. Richetti A, Cavallaro A, Ainis T, Fimiani V. Effect of mycotoxins on some activities of isolated human neutrophils. Immunopharmacol Immunotoxicol 2005;27:433-46.
11. Schlatter J. Toxicity data relevant for hazard characterization. Toxicol Let 2004;153:83-9.
12. Froquet R, Sibiril Y, Parent-Massin D. Trichothecene toxicity on human megakaryocyte progenitors (CFU-MK). Hum Exp Toxicol 2001;20:84-9.
13. Vlata Z, Porichis F, Tzanakakis G, Tsatsakis A, Krambovitis E. A study of zearalenone cytotoxicity on human peripheral blood mononuclear cells. Toxicol Lett 2006;165:274-81.

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