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Haematological Changes in Citrinin-Administered Mice, *Mus musculus*

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Oral administration of 50 ppm of citrinin to young weaning mice caused a fall in the haemoglobin content and RBC count. The total WBC-count increased, mainly due to increase in the number of lymphocytes. The neutrophil count, however, showed a decline. Treated animals showed a rise in the levels of blood-sugar and blood-urea. Empty stomach administration had more acute and toxic effects.

Key Words: Citrinin-administration, *Mus musculus*, **Blood sugar, Blood urea**

Introduction

Citrinin is a common mycotoxin elaborated by *Penicillium citrinum*. High levels of citrinin are reported in food and feed. Therefore, this mycotoxin is a great health risk for human beings and domestic animals (Scott et al. 1972). Few reports are available on the histopathology of liver, kidney and gall bladder caused by citrinin (Wyllie & Morehouse 1978, Vesela et al. 1983, Kanisawa 1984). In the present communication the effect of citrinin on blood histology as well as on levels of blood-sugar and blood-urea is reported.

Materials and Methods

Six-week old 36 albino Swiss mice (*Mus musculus*) of body weight 25 to 30 g were categorized in three groups, each comprising 12 animals (6 males + 6 females). The first group of the animals were administered with 0.25 ml of 50 ppm pure citrinin solution by intubation at about 8.00 am and the regular meal was supplied to them an hour thereafter. The second group was given the same amount of toxin (per animal per day) but by mixing it with their standard sterilized food ("*Gold Mohur*" mice feed, Hindustan Lever). The third group of the animals did not receive any toxin, and served as control. The dose 50 ppm was selected because of the fact that it is in such low doses that this toxin is present in our food and feed (Nishijima 1984).

The animals were maintained under non-germ free conditions. Appropriate care and hygienic conditions were maintained in order to keep them healthy and free from any apparent infection. Each group was kept in separate cages at room temperature. Blood was collected from the tail tip after 30, 60 and 90 days of the treatment and analysed for histological details. After 120 days, the blood was collected directly from the heart through syringe for estimation of blood-sugar and blood-urea.

Total counts of RBC were estimated using the haemocytometer, and haemoglobin content was determined by Hellige method (D' Amour et al. 1965). Differential count of leucocytes was done by Leishman staining (Wintrobe 1962). Blood-sugar was estimated by titrimetric method for true sugar (Wootton 1964) and glucose oxidase method (Plummer 1978). Blood-urea was determined by Biacetyl and Nessler methods (Wootton 1964).

The values so obtained were put to t-test for evaluating the significance of the difference between the control and the treated variants, and also between the two treated variants. As age is well known to affect these haemotological parameters, concurrent controls were invariably maintained and the comparisons were made only with the values obtained from control group of animals belonging to the corresponding age-group.

Results and Discussion

There was a significant fall in the level of haemoglobin content and total count of RBCs after citrinin treatment (table 1). The number of leucocytes increased which was obviously due to increase in the number of lymphocytes; the proportion of neutrophils decreased upon treatment (table 2). Direct administration of the toxin appears to cause more acute changes, but it is significantly so only in the haemoglobin content on the ninetieth day, and in lymphocyte and neutrophil counts on the thirtieth day (tables 1 and 2). Reduction in haemoglobin content is possibly due to reduction in total count of RBC, because intraerythrocytic depletion in its content in form of haemoglobinopathies was not observed.

Table 1 *Means ($\bar{X} \pm SE$) of the haemoglobin content, total counts of RBC and WBC of the whole blood in control and citrinin-treated mice*

Parameter	Day of observation	Mode of administration		
		Control	Citrinin with food	Citrinin independent
Haemoglobin content (g/100 ml of blood)	30th	12.5 ± 0.1	11.3 ± 0.2*	11.0 ± 0.1*
	60th	12.5 ± 0.1	10.2 ± 0.5*	9.4 ± 0.2*
	90th	12.5 ± 0.1	9.4 ± 0.2*	7.3 ± 0.2*#
Total count of RBC (in $10^6/mm^3$)	30th	5.4 ± 0.06	4.34 ± 0.06*	4.28 ± 0.07*
	60th	5.4 ± 0.06	4.1 ± 0.05*	4.08 ± 0.04*
	90th	5.4 ± 0.06	3.9 ± 0.25*	3.9 ± 0.25*
Total count of WBC (in $10^3/mm^3$)	30th	8.2 ± 0.04	8.7 ± 0.04*	9.36 ± 0.62*
	60th	8.04 ± 0.06	9.26 ± 0.02*	10.5 ± 0.4*
	90th	8.27 ± 0.35	9.95 ± 0.45*	10.81 ± 0.41*

*indicates significant difference (at 5% level) with the corresponding value in the control
#indicates significant difference between two treatment variants

Citrinin is reported to interfere with the absorption of digested food through the gastrointestinal tract. The anemia in the treated animals may be a consequence of such citrinin-impaired absorption process. Perhaps this is why the direct administration of the citrinin (in empty stomach condition) caused greater inhibition to produce more severe effects than the one administered along with the food.

The increased lymphocyte count among the treated animals is suggestive of the induction of some pathology (as in liver and kidney; earlier reported by Wyllie & Morehouse 1977) by citrinin. The decrease noted in neutrophil count may be the result of direct toxic effect on bone marrow and/or their rapid

Table 2 *Differential count of WBC in the whole blood of control and citrinin-treated mice. Result expressed in mean ($\bar{X} \pm SE$) number per 100 WBC*

Days of observation	WBC cell types	Mode of administration		
		Control	Citrinin with food	Citrinin independent
30th	Lympho	45 ± 2.4	56 ± 2.1*	70 ± 3.2*#
	Neutro.	52.3 ± 2.7	42 ± 3.2	28 ± 1.7*#
	Mono.	2	1	1
	Eosino	1	1	1
	Baso.	—	—	—
60th	Lympho	52.3 ± 2.7	65 ± 1.8*	72 ± 1.3
	Neutro.	46.0 ± 1.3	32 ± 4*	25.8 ± 26*
	Mono.	1	2	2
	Eosino.	1	1	1
	Baso.	—	—	—
90th	Lympho.	58 ± 2.2	68 ± 1.8*	77 ± 1.3*
	Neutro.	40.6 ± 1.6	29.0 ± 2.6*	21.8 ± 1.8*
	Mono.	1	1	0.9
	Eosino.	1	1	1
	Baso.	—	—	

*indicates significant difference (at 5% level) with the corresponding value in the control
#indicates significant difference between two treatment variants

Table 3 *Effects of citrinin on blood sugar and blood urea levels in mice*

Sex	Group	Glucose level (mg/100 ml of blood) (Mean ± SE)	Urea content (mg/100 ml of blood) (Mean ± SE)
Male	Control	78 ± 0.83	20.2 ± 0.82
	Citrinin with food	155 ± 4.08*	29.9 ± 0.80*
	Citrinin independent	190 ± 1.63*	40.2 ± 0.65*
Female	Control	76 ± 0.82	19.5 ± 0.2
	Citrinin with food	145 ± 4.08*	28.1 ± 0.15*
	Citrinin independent	161 ± 0.82*	37.1 ± 0.43*

*indicates significant difference (at 5% level) with the corresponding value in the control

destruction in peripheral blood by hypersensitivity reactions (Gruch 1960) or hypersplenism. The increased lymphocyte and decreased neutrophil counts in controls from 30 through 90 days (table 2) is attributed to ageing.

As mentioned earlier, the amount of glucose and urea present in blood of the animals was estimated on the 90th day of the treatment. The data obtained show that the levels of both the chemicals increase (table 3) in either variant of the treatment, which is suggestive of the hepatotoxic and nephrotoxic nature of citrinin or of its derived form(s). For these two parameters, the males appeared to be more susceptible than females; sex-influenced variations were not observed for the remaining parameters.

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