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Residual Ochratoxin-A in Rabbit Tissues Following Experimental Intoxication

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The residual effects of OT-A was evaluated in eight weeks old, New Zealand White rabbits, weighing 350 to 400 gm. Continuous feeding of OT-A @ 1000 and 2000 ppb, resulted in accumulation and building up of OT-A concentration in different tissues with highest toxin concentration in blood followed by kidneys, liver and muscles. In the follow-up experiment, OT-A residue was detected in the tissues even up to 4 weeks after withdrawal from diet following 3 weeks exposure.

KEYWORDS
Ochratoxin- A, residues, rabbit.

INTRODUCTION

Rabbits are highly susceptible to ochratoxicosis (1) and pharmacokinetic studies in rabbits (2) indicate wide ochratoxin-A (OT-A) distribution in the body. However, residual effects in rabbit tissue have not been studied. Present study describes the residual effects of ochratoxin-A (OT-A) in rabbit tissues following experimental intoxication.

MATERIAL AND METHODS

OT-A was produced by culturing *Aspergillus ochraceus* NRRL-3174, on maize, earlier confirmed to be free of OT-A and aflatoxins (3). After establishing the toxin levels by thin layer chromatography (TLC), cultured maize was mixed with rabbit feed so as to make a final concentrations of 1000 and 2000 ppb. Eight weeks old, New Zealand White rabbits, weighing 350 to 400 gm, were procured from the Laboratory Animal Resources, IVRI and allotted randomly to three groups- I, II and III. Group-I, consisting of 6 animals, was fed control diet tested to be free of AF-B1 and OT-A. Group-II and -III, containing 12 animals each, were maintained on 1000 and 2000 ppb OT-A diets respectively. The animals were sacrificed by ex-sanguination on days 15, 30, 45 and 60 and the toxin concentration was analyzed in the blood, liver, kidney and muscles (skeletal + stomach + cardiac). OT-A was extracted from the tissues adopting Van den Molen et al (4) method.. The method was standardized for small samples, as per experimental requirements, by carrying out spiking experiments. The plasma / serum samples were pre-cleared by centrifugation at 2-3 x 10^3 rpm for 10 min. and the toxin extracted by the method described by Mortensen et al (5). OT-A levels were estimated by TLC and UV-spectrophotometry at 333 nm in ethanol ($E^{1\%}_{1cm}$ ~ 6100) (6). In a follow up experiment, the toxin diet was withdrawn in experimental rabbits (10 in each 1000 and 2000 ppb OT-A groups) after 3 weeks of continuous feeding and tissue analyzed for residual OT-A up to 4 weeks post withdrawal.

RESULTS

In the present study quantitative determination of toxin (OT-A) levels in the tissues (blood, kidneys, liver and muscles) at days 15, 30, 45 and 60, of feeding 1000 and 2000 ppb OT-A in the diet revealed that the toxin levels were highest in the blood followed by the kidneys and liver. Low

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levels of toxin were found in the muscles [Table 1]. Further higher levels of OT-A were observed in the 2000 ppm OT-A group rabbits when compared with that of 1000 ppm OT-A group in a time related fashion. Residual toxin was detected even after 4 weeks post withdrawal of OT-A. in the kidneys, liver and muscles of rabbits fed OT-A continuously for 3 weeks,

DISCUSSION

OT-A has been reported to have high affinity for plasma albumin (7) that favours its wide distribution in the body. Although, auto-radiographic studies in the pregnant mice (8) have revealed highest tissue concentration of OT-A in the liver followed by kidneys, blood and other tissues, yet, our results were consistent with those reported in fowl, turkeys, quail, pigs and rats (9-12). The highest levels of toxin residues in the kidney and liver might be explained on the basis of more blood circulation in these organs as well as to the high affinity of OT-A especially for the proximal convoluted tubules in the kidney (10). Also in the liver OT-A has been reported to diffuse into the bile from the blood against concentration gradient (13) and the damage to the biliary ductules might have further added in the retention of the toxin. The time related increase in the residual toxin concentration in the tissues and the detection of toxin in the kidneys, liver and muscles even after 4 weeks of withdrawal suggested the persistent and cumulative effects and slow metabolic clearance from these tissues. Resorption of OT-A and its metabolites in kidneys as well as entero-hepatic recirculation (14) might also be partly responsible for persistence of OT-A in tissues. The observation of higher levels of OT-A residues in the 2000 ppb OT-A group is consistent with the earlier reports of good correlation between OT-A concentration in the feed and its residues in the kidney ($r = 0.82$), serum ($r = 0.78$) and other tissues (15). The present finding gains an immense significance, not only from the management point of view but also because of public health considerations, due to the presence of considerable amounts of OT-A in the edible tissues of rabbits, especially when OT-A is known to have immunosuppressive, teratogenic and carcinogenic potentials (13) and is a possible geno-toxin. Hence, detailed studies are warranted to evaluate the permissible OT-A levels in the animal and human food.

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TABLES

Table 1: OT-A Concentration (ng / gm, ppb) in different tissues of OT-A fed (1000 and 2000 ppb) rabbits. (Mean±S.E)

Tissues	**OT-A in Feed (ppb)**	**Period of feeding OT-A diets (days)**				**Overall**
		15	**30**	**45**	**60**	
Blood	1000	45.00 ± 15	61.50 ± 16.5	72.50 ± 12.5	70.00 ± 5.0	62.25 ± 6.38
	2000	95.00 ± 15.0	76.00 ± 11.0	105.00 ± 10.0	91.50 ± 13.5	91.87 ± 6.16
Kidney	1000	Traces- 15.4	13.65 ± 3.85	16.85 ± 3.35	14.40 ± 4.1	Traces – 20.20
	2000	10.10 ± 3.7	22.95 ± 0.55	21.35 ± 0.85	19.60 ± 3.80	18.50 ± 2.14
Liver	1000	1.0 ± 0.60	2.25 ± 0.55	2.85 ± 0.55	2.60 ± 0.20	2.17 ± 0.65
	2000	2.00 ± 0.40	3.05 ± 0.35	3.45 ± 0.15	3.50 ± 0.30	3.00 ± 0.25
Muscles (skeletal + stomach + cardiac**)**	1000	(Traces)	(Traces – 0.5)	0.60 ± 0.10	(Traces – 0.40)	(Traces – 0.70)
	2000	(Traces)	(Traces)	0.65 ± 0.25	0.60 ± 0.10	(Traces – 0.90)