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Effect of Low Protein Diet on the Acute Doses of Actellic-20 in the Heart And Brain of Albino Rats

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Abstract: A study on the effect of low- protein diet on the toxicity of acute doses of Actellic-20 in the heart and brain of albino rats was carried out. Three weeks old weaning albino rats *Rattus norvegicus* were grouped into two and fed low and normal protein diet for four weeks after which they were given Actellic-20 at low dose levels (100ppm and 150ppm) by oral intubation. The rats were sacrificed at 24h, 72h, and 120h after dosing and the activities of Alkaline phosphatase, Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST) were monitored in these tissues. The activities of Alkaline phosphatase, Alanine aminotransferase and Aspartate aminotransferase were significantly reduced ($P<0.05$) at 24h and 120h but significantly increased at 72h in both animals fed low protein and normal protein diet dosed with Actellic-20 when compared with the control rats. It is considered that effect of this pesticide Actellic-20 on the activities of these enzymes on the heart and brain may affect energy production and amino acid metabolism in these organs.

Key words: Albino rats, low protein diet, actellic-20

Introduction

Pesticides retain their bioactivity for long period of time and represent a potential hazard to non-target organisms (like human and other animals), though they are employed for crop protection, storage and transport of food. They are frequently used in areas where population depend on nutritionally inadequate diets thus posing additional problem (WHO, 1990).

Actellic-20 is an organophosphorus pesticide, which has a molecular weight of 305 ($C_{11}H_{20}$ N_3O_3PS), its boiling point is below 100°C and melting point of 15-18°C. It is soluble in water at 30°C and miscible in all proportions with organic solvents. It is often used in form of powder to store grain (maize, beans, rice and wheat) (USEPA, 1988).

Adequate nutrition is an important factor influencing the ability of an organism to handle environmental chemicals (Newborne, 1975). However, protein malnutrition is a major public health problem in some parts of the world, including Nigeria and the West African subregion (FAO, 1986). There appeared to be an optimal protein intake necessary to protect the pesticide tolerance in rats, this being approximately certain percentage in the feed. Beyond this protein level the induced pesticide toxicity was on the increase (Boyd and Krijnen, 1969; Boyd *et al*., 1970). It has been shown that LD_{50} of pesticide is altered by the quality and quantity of diet (Boyd and Krupa, 1970). Malnourished human population may be more prone to the toxic effects of these pesticides. The clinical features of protein energy malnutrition are consequences of insufficient supply of energy and amino acid to the tissues which need them for protein synthesis (Conney *et al*., 1996) There is paucity of information in recent times on the possible adverse effects this pesticide could have on farmers subsisting on sub-optimal or normal levels of protein.

In this study, the effects of acute doses of Actellic-20 on animal fed both low and normal protein diet on the heart and brain being vital organs affected in protein deficiency were investigated.

Table 1: Composition of diets

Component	Low Protein Diet (%)	Normal Protein (%)
Carbohydrate	82	59
Casein	3	26
Fat	8	8
Minerals	4	4
Vitamins	3	3

Materials and Methods

Animal treatment: A total of 28 female weanling rats of about four weeks old were obtained from the Animal House of Biochemistry Department, University of Ilorin, They were randomly assigned to cages, acclimatized for 24hrs before introduction of the diet. Water and diet were given *ad libtum*. The rats were divided into four groups i.e 2 test groups and 2 control groups (Table 1).

Control 1 and 2 rats were fed with formulated control diet containing normal protein level. Test 1 and 2 rats were fed with low protein diets. Animals were weighed weekly. After four weeks of feeding, Test one group of rats were given Actellic-20 at dose level of 100mg/kg and Test two group of rats were given 150mg/kg dose. Control one rats were subdivided into two, and dosed with Actellic-20 at 100mg/kg and 150mg/kg respectively. Control two rats were not dosed at all.

Rats from each group were sacrificed under

Table 2: Activities of aspartate aminotransferase in the heart and brain of rats

	Heart			Brain		
	24h	72h	120h	24h	72h	120h
Test 1[A]	1.50±0.01	6.88±0.17	2.61±0.03	2.39±0.01	3.58±0.14	1.55±0.07
Control 1[A]	1.54±0.07	4.83±0.03	2.61±0.38	2.84±0.01	3.03±0.23	3.68±0.0
Test 1[B]	1.60±0.03	6.97±0.13	4.33±0.45	5.16±0.13	4.05±0.11	3.91±0.01
Control 1[B]	1.63±0.51	2.97±0.10	2.06±0.61	3.43±0.11	5.19±0.45	3.68±0.02
Control 2	3.81±0.01	3.81±0.01	3.81±0.01	9.28±1.35	9.28±1.35	9.28±1.35

Test1[A] and Control 1[A] were given 100 mg/kg dose of Actellic-20. Test 1[B] and Control 1[B] were given 150mg/kg dose of Actellic-20
Values are means of five determinations ± SD

Table 3: Activities of alanine aminotransferase in the heart and brain of rats

	Heart			Brain		
	24h	72h	120h	24h	72h	120h
Test 1	[A]0.54±0.01	5.53±0.75	2.61±0.21	0.42±0.02	3.98±0.15	2.90±0.05
Control 1[A]	0.38±0.03	2.47±0.43	1.83±0.13	0.73±0.05	3.69±0.72	1.81±0.11
Test 1[B]	0.51±0.07	2.99±0.25	3.08±0.25	1.13±0.07	4.55±0.18	2.58±0.21
Control 1[B]	0.66±0.11	3.76±0.13	1.90±0.15	1.07±0.04	3.34±0.52	2.00±0.18
Control 2	1.21±0.11	1.21±0.11	1.21±0.11	1.8±0.03	1.8±0.03	1.8±1.0.03

Test1[A] and Control 1[A] were given 100 mg/kg dose of Actellic-20. Test 1[B] and Control 1[B] were given 150mg/kg dose of Actellic-20
Values are means of five determinations ± SD

anaesthesia by cervical dislocation at 24, 72, 120h interval. All reagents used were of analytical grade and products of BDH chemical, England.

Tissue preparation: The heart and brain were quickly removed drained of blood, weighed and put into ice cold 0.25M sucrose buffer solution. They were then homogenized in ice-cold 0.25M sucrose buffer solution (1:4w/v) Triton X – 100 was added to a final concentration of 1%. The homogenates were frozen overnight before enzyme assay.

Enzyme and protein measurements: Aspartate animotransferase (E. C. 2:6:1:1) and Alanine aminotransferase (E. C. 2:6:1:2) activities were determined, using appropriate buffer system by measuring the pyruvate resulting from the transamination reactions at 546nm (Reitman and Frankel, 1956). Protein concentration was measured by the biuret method (Plummer, 1978).

Alkaline phosphatase (ALP) activity was measured by the method of (Wright *et al*., 1972) where p-nitrophenyl phosphate was hydrolyzed and the absorbance read at 400nm.

All measurements were done using spectronic-20 Statistical comparison of results was made using Analysis of variance, and p-values < 0.05 were regarded as significant.

Results and Discussion

Physical observation made during the experiment, revealed loss of hair in rats fed with low protein diet before oral intubation this might be due to lack of synthesis of keratin. Watery stool or diarrhoea was also observed within the first two weeks of feeding in the rats fed low protein diets. These observations agrees with previous report (Tayiro, 1981).

The result in Tables 2-4 showed that in both the heart and the brain, the specific activities of all the enzymes assayed increased significantly at 72h and decreased significantly at 24h and 120h for both groups of animals. The reason might be that at 24h, the acute effect of the pesticide reduced the level of these enzymes at 72h the body produced more enzyme to replace the lost ones and at 120h, it could be that the pesticide reduced the activities of the enzymes.

The specific activities of Aspartate aminotransferase (Table 2) in the brain of animals fed low protein and normal protein dosed with Actellic-20 showed significant ($P<0.05$) decrease when compared with control 2 at 24h, 72h and 120h, this might be due to the acute effect of actellilc-20 since its lipophilic and the brain is mainly made up of lipids, it will easily diffuse through the membrane. This may lead to the destruction of brain cells and release of the enzyme into extracellular compartment. This might grossly affect amino acid metabolism in the brain (Marcus *et al*., 1979).

Also in the heart significant increase ($0<0.05$) in the activities of the enzyme was observed at 24 and 72h but the activities tended toward that of control two at 120h and this might be due to detoxification of the pesticide. It had been reported that Actellic 20 is rapidly excreted (USEPA, 1988).

The activities of alanine aminotransferase (Table 3) decreased significantly ($p<0.05$) when compared to control two at 24h, but at 72h and 120h, significant increase was observed this might be due to increased synthesis which may arise from an attempt to repair

Table 4: Activities of alkaline phosphatase in the heart and brain of rats

	Heart			Brain		
	24h	72h	120h	24h	72h	120h
Test 1[A]	7.70±0.26	66.46±2.5	15.22±0.05	2.0±0.02	14.98±0.45	2.47±0.05
Control 1[A]	5.49±0.13	47.33±1.1	18.50±0.01	2.2±0±0.10	10.11±0.23	5.89±0.20
Test 1[B]	2.90±0.66	68.64±2.8	11.60±0.58	1.13±0.07	4.55±0.18	2.58±0.21
Control 1[B]	6.35±0.04	55.112±0.9	14.15±0.02	2.10±0.08	8.24±0.11	5.59±0.40
Control 2	20.23±0.05	20.23±0.05	20.23±0.05	6.83±0.13	6.83±0.13	6.83±1.0.13

Test 1[A] and Control 1[A] were given 100mg/kg dose of Actellic-20. Test 1[B] and control 1[B] were given 150mg/kg dose of Actellic-20
Values are mean of five determinations ± SD

possible damage to the heart by pathology of the pesticide. Elevated levels of aspartate aminotransferase have been used as indication for some forms of hepatic diseases (David and Johnston, 1999) such as hepatitis and necrosis as well as myocardial infarction.

Alkaline phosphatase is a marker enzyme for the plasma membrane. The decrease in activity observed in this study (Table 4) at 24h in the heart and brain could be due to disruption of the plasma membrane leading to leakage of the enzyme into the extracellular fluids which might grossly affect the rate of hydrolysis of phosphoric group of esters and hence production of energy in the form of ATP.

However, at 72h there was an increase in the activity of this enzyme in both organs this might be due to *denovo* synthesis. When the level of dietary protein is reduced, the basal rate of the cytochrome P_{450} the enzyme responsible for detoxification is diminished. The rate of metabolic processes is also affected because these processes are catalyzed by enzymes, and regulated by hormones which are not made available sufficiently in this deficient state (Veena and Murthy, 1994). The toxic manifestations could also be due to reduced synthesis of albumin (Maurice, 1994). Consequently these animals may have a greater amount of ingested pesticide in its free form than if there is albumin available to which it could bind. Since membranes are made up of proteins this diet could have weakened the structural integrity of these organs in rats fed low-protein diet leading to leakage of enzymes to extracellular fluids However, for the rats maintained on normal protein diet, the significant difference ($P<0.05$) herein observed compared to the control (that was not dosed) may be due to the lipophilic nature of this pesticide. This may then affect the structural architecture of the membrane as reflected in the activities of the enzyme in these tissues.

From this experiment it is clear that the activities of these enzymes in the brain and heart of animals in both the low protein and normal protein groups (i.e. control one) were affected by acute doses Actellic-20 except that the effect on the test groups was more pronounced compared to control one group. Since changes in dietary protein affected the physiological state of rats, farmers working with pesticide should be mindful of their exposure level to this chemical and should take a normal protein diet, which might be helpful in combating the detrimental effects of the pesticide.

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