J.Biol.Control, 4 (1), 35-39, 1990

Safety of Two Granulosis Viruses Infecting Sugarcane Borers to Albino Rats

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ABSTRACT

Male and female albino rats fed with two granulosis viruses, one infecting sugarcane shoot borer, *Chilo infuscatellus* snell. and the other infecting internode borer, *C. sacchariphagus indicus* (Kapur) at a dose equal to $100x2.5x10^{14}$ inclusion bodies/75 kg man did not show any mortality, abnormality in general behaviour, food consumption, body weight gain, gross necropsies, clinical chemistry and haematological examinations. When the rats were injected with the viruses intraperitoneally at 1.1×10^{12} inclusion bodies/rat (average body weight 80.7 g) also, no harmful effect could be detected.

Key Words : Granulosis virus, safety, Albino rats

Granulosis viruses (GV) are reported to infect the sugarcane shoot borer, *Chilo infuscatellus* Snell. (Easwaramoorthy and David, 1979) and the internode borer, *C. sacchariphagus indicus* (Kapur) (Crambidae : Lepidoptera) (Mehta and David, 1980). Both the viruses are active throughout the year among the host insect populations (Easwaramoorthy, 1984) and are widely distributed in the sugarcane ecosystem (Easwaramoorthy and Jayaraj, 1987a). Detailed studies carried out with the viruses indicate that the GV infecting shoot borer is effective in the control of the pest under field conditions (Easwaramoorthy and Santhalakshmi, 1988). Though the specificity of GVs to their insect hosts and their safety to higher animals are known (Ignoffo, 1973; Burges, 1980a), so far no study has been carried out with GVs of sugarcane borers. Information on their safety to other non-target organisms is necessary before large scale field use of the viruses is taken up. So the present study was carried out to determine the acute toxic effects of the viruses to albino rats by oral feeding and intraperitoneal injection methods.

MATERIALS AND METHODS

The viruses were purified by 30-65% (W/W) continuous sucrose- gradient centrifugation (Harrap and Longworth, 1974). The purified inclusion bodies of the viruses were concentrated in sterile normal saline (0.85% sodium chloride). Prior to use, the number of bacterial cells or spores and presence or absence of coliforms were determined. To find out the bacterial population, differential bacterial plate counts were made. The number of bacterial cells present was estimated by pour plate technique using nutrient agar medium and the number of viable spores present was determined by pasteurization technique. MacConkey's agar medium was used to estimate the coliforms present in the virus preparations following standard microbiological techniques (Pelczar,1957).

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Oral Feeding Test

Five male and five female albino rats of three to four weeks age with an average body weight of 68.7 g, obtained from the department of Biochemistry, Tamil Nadu Agricultural University, were randomly assigned to each of the three treatments. The dose fed to the experimental animals was calculated at 100 times the average field dose per 0.4 ha with a conversion ratio of the weight of the test animal to the weight of man. Assuming that a 75 kg man would be exposed to 100 x 2.5 x 10^{14} inclusion bodies (40 ha dose), the dose per kg of body weight was worked out. The experimental animals were then fed with the same dose adjusted to their body weights using an one ml tuberculin syringe fitted with five cm tefflon sleeve of 0.5 ml diameter. Control rats were fed with equal quantity of sterile normal saline. The animals were maintained in metabolic cages and known quantity of standard commercial food was supplied twice a day and water *ad libitum*. The test was terminated after 28 days.

The animals were examined daily for their general behaviour and for the appearance of any signs of toxicity. The food consumption was recorded daily and weight gain and body temperature at weekly intervals. The food consumption index was worked out. At the termination of the experiment, the rats were etherised and blood withdrawn by cardiac puncture method for various clinical analyses. Wet weights of important organs like heart, liver, lungs, spleen, kidney, testes and ovaries were recorded and histopathological observations were made. Blood glucose and serum protein were estimated following the methods of Somogyi (1952) and Lowry *et al.* (1951), respectively. Serum glutamic-pyruvic transminase and serum glutamic oxaloacetic transminase were estimated following the method of Mohun and Cook (1957). Blood haemoglobin was estimated using Sahil's haemoglobinometer.

Intraperitoneal injection test

Five female rats of four weeks age (average body weight 80.7g) were used per treatment in this test. The viruses prepared as outlined under acute toxicity test were suspended in Freund's complete adjuvant and 0.5 ml of phosphate buffer at a pH of 7.5. Each rat was injected with a total volume of 0.5 ml of the virus buffer mixture or the buffer mixture alone. The dose of the viruses used in the study was 1.1 x 10^{12} inclusion bodies/rat. The rats were maintained in metabolic cages and data collected as described under acute oral toxicity test.

RESULTS AND DISCUSSION

Throughout the course of the experiment all the rats in the three treatments were healthy, and no abnormality could be detected in their bodily appearance and behaviour. The gain in body weight varied from 95 to 102.8 g in males and 73.5 to 81.0 g in females and the differences due to treatments were not

Table 1. Body weight, food consumption and temperature of virus fed and healthy albino rats

Observations	Shoot borer virus fed		Internode borer virus fed		Control	
	Male	Female	Male	Female	Male	Female
Initial weight (g)	69.3	68.9	74.2	68.4	75.3	74.2
Final Weight (g)	164.3	145.5	177.0	149.4	171.3	147.7
Weight gain (g)	95.0	76.6	102.8	81.0	96.0	73.5
Total food consumed (g)	277.1	236.8	298.9	261.9	259.8	239.0
Consumption index (C.I.)	0.34	0.32	0.34	0.31	0.37	0.31
Mean body temperature (°C)	37.9	38.0	38.0	38.0	37.5	38.0

Table 2. Fresh weight of organs(g) of virus fed and healthy albino rats

Organ	Shoot borer virus fed		Internode borer virus fed		Control	
	Male	Female	Male	Female	Male	Female
Liver	7.8±0.9	5.8±0.7	8.8±0.8	5.9±0.4	7.2±0.6	5.1±0.5
Kidney	1.9±0.3	1.4±0.2	2.0±0.4	1.4±0.3	1.9±0.4	1.3±0.2
Spleen	0.4±0.1	0.3±0.1	0.4±0.1	0.3±0.1	0.4±0.1	0.3±0.1
Lungs	0.9±0.2	0.9±0.1	0.9±0.3	0.8±0.2	0.9±0.2	0.6±0.3
Heart	0.5±0.1	0.4±0.1	0.5±0.1	0.4±0.2	0.5±0.1	0.3±0.1
Testes	2.7±0.4	—	2.8±0.6	—	2.5±0.4	—
Ovaries	—	1.8±0.7	—	1.6±0.3	—	1.2±0.4

significant (Table 1). There was no significant variation in food consumption index and mean post - treatment body temperature.

In any of the rats dissected out for necropsy, no abnormality was detected. In general, the weights of the body organs were proportional to the overall body weight of rats at the time of sacrifice (Table 2). There was no appreciable difference in the weights of the different organs of the treated and control rats. Histological examination of all the tissues showed no evidence of tissue damage in virus-fed animals.

The amount of glucose in blood was 186.5, 193.8 and 179.5 mg in males and 218.5, 229.5 and 215.3 g per 100 ml of blood in females in shoot and internode borer GV treatments and control, respectively. There was also no appreciable variation in serum protein, serum enzymes, plasma volume and haemoglobin content (Table 3).

No death occurred in any of the virus or saline injected rats. Body temperature, body weight gain and feed efficiency of GV injected rats were essentially similar to those in control. In addition, no difference in response to virus could be detected in gross necropsy, clinical chemistry and haemotology examinations (Table 4).

The above results indicate that the GVs infecting shoot borer and internode borer of sugarcane are harmless to albino rats. Only a few reports are available on the safety of GVs to higher animals. Ignoffo (1968) reviewed the work on specificity of insect viruses and reported that the six GVs tested against various

Table 3. Biochemical constituents in virus fed and healthy albino rats

Observations	Shoot borer virus fed		Internode borer virus fed		Control	
	Male	Female	Male	Female	Male	Female
Blood glucose (mg/100ml)	186.50	218.50	193.80	229.50	179.50	215.30
Serum protein (g/100ml)	2.32	3.41	2.44	3.36	2.35	3.35
Serum glutamic pyruvic transaminase (mg/100ml)	33.33	35.92	33.33	31.42	32.00	33.62
Serum glutamic Oxaloacetic transminase (mg/100ml)	74.26	73.47	79.62	75.34	72.47	73.56
Plasma volume (%)	49.20	49.70	50.60	48.60	51.30	48.30
Haemoglobin (g/dilution)	12.30	11.80	9.30	9.70	9.10	9.60

Table 4. Results of safety tests with albino rats by intra peritoneal injection method

Observations	Shoot borer virus injected	Internode borer virus injected	Control
Initial weight (g)	66.97	71.00	63.67
Final weight (g)	136.00	130.17	131.33
Weight gain (g)	66.33	59.17	67.66
Total food consumed (g)	255.18	212.74	237.08
Consumption index (C.I.)	0.26	0.28	0.29
Mean body temperature (°C)	37.8	38.0	37.9
Organs fresh weight (g)			
i) Liver	5.69	6.66	5.75
ii) Kidney	1.60	1.59	1.44
iii) Spleen	0.36	0.37	0.33
iv) Lungs	0.92	0.93	0.88
v) Heart	0.35	0.46	0.38
Blood glucose (mg/100 ml)	228.3	235.6	208.0
Serum protein (g/100 ml)	3.44	3.51	3.35
Serum glutamic-pyruvic transaminase (mg/100 ml)	33.33	31.46	32.50
Serum glutamic-oxaloacetic transaminase (mg/100 ml)	73.58	77.23	71.46
Plasma volume (%)	50.6	48.9	49.3
Haemoglobin (g/dilution)	10.6	11.1	11.0

vertebrate species did not show any demonstrable case of virus toxicity, pathogenicity and allergenicity. Later, the GVs infecting *Cydia pomonella* (L.) (Groner *et al.*, 1978), *Estigmene acrea* (Dru.) (Heimpel, 1971) and *Pieris rapae* (Linn.) (Anon.,1980) were also found harmless to higher animals like mice, guinea pigs, pig, cow, lamb, etc., by various methods of administration.

According to Burges *et al.* (1980a), less is known about the safety of GVs than nuclear polyhedrosis viruses (NPVs), the other group of insect viruses included under Baculoviridae. On the basis of present evidence and the fact that the GVs are found only in the order Lepidoptera with a narrow host range than NPVs, GVs can be deemed as safe as NPVs (Burges *et al.*, 1980 b) which are commercially exploited as microbial pesticides.

The GVs infecting shoot and internode borers of sugarcane were also found safe to some common parasites and predators occurring in the sugarcane ecosystem (Easwaramoorthy and Jayaraj, 1987b), silkworms (Easwaramoorthy and Jayaraj, 1988) and honey bees (Easwaramoorthy and Jayaraj, 1987c) and hence can be utilised in fields without affecting the non-target organisms.

ACKNOWLEDGEMENTS

The authors are thankful to Dr.K.Mohan Naidu, Director and Dr.H.David, Head, Division of Entomology, Sugarcane Breeding Institute, Coimbatore for the facilities provided. Sincere thanks are also due to Mrs.K.Parvathy, Assistant Professor of Biochemistry, Tamil Nadu Agricultural University for the valuable help rendered.

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