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ORIGINAL ARTICLE

Effect of azadirachtin of neemix-4.5 on SWR/J mice

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Toxic effect;
Neem-based insecticide

Abstract Inbred normal SWR/J male and female mice, 8–10 weeks old and weighing 22.55–26.72 g, were used throughout the study. A total of 100 males and 100 females were used and were divided into 20 groups, 10 animals in each group.

Azadirachtin of neemix-4.5, a commercial botanical pesticide derived from the neem tree, orally administered to male and female SWR/J mice at a dose level 9.0 mg/kg (1/10 LD_{50}) for different treatment periods (2, 4, 6, 8 or 11.5 weeks) has produced signs of toxicity, mortality and changes in body and tissue weights of both sexes at almost all treated periods used in the present study. Moreover the oral administration of this dose level for 11.5 weeks has also resulted in some histopathological changes in the livers, kidneys and testes of treated animals compared with the control group, and the degree of these changes ranged from mild to severe in these organs of treated males. However, conflicting results have been reported concerning the toxicity of azadirachtin in mammalian species using different formulations of neem-based pesticides. It appears, therefore, that the toxicity produced by neemix-4.5 in the present study may be due to factors other than azadirachtin in this formulation.

1. Introduction

Increasing concern about pesticide accumulation in the environment stimulates search for natural compounds that could replace synthetic insecticides in insect pest control (Adel and Sehnal, 2000). Neem is the most promising potential source of biopesticide of botanical origin (Schmutterer, 1995; Raizada et al., 2001). During the past two decades, neem seeds (*Azadirachta indica*, A. Juss) has gained increasing attention as a natural insecticide, and its activity has been evaluated against many economically important insect species (Schmutterer, 1990; Hashem et al., 1998; Kreutweiser et al., 2002; Liang et al., 2003; Charleston et al., 2005).

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Nomenclature

S	spermatozoa	Pr	proximal convoluted tubule
A	artery	Si	sinusoid
K	Kupffer cells	Sp	spermatocytes
D	damaged spermatocytes	Ic	interstitial cells
I	interstitial space	L	lumen
H	hepatocyte	G	glomerulus
N	nucleus	Di	distal convoluted tubule

Neem-based insecticides have deterrent, antiovipositional, antifeedant, growth-regulating, fecundity- and fertility-reducing properties on insects (Mordue and Blackwell, 1993; Hashem et al., 1998). The principle insecticidal component of neem extracts is the limonoid, azadirachtin. Azadirachtin has been effectively used against more than 400 species of insects and has proved to be one of the most promising plant ingredients for integrated pest management at the present time (Isam, 1999; Walter, 1999; Saber et al., 2004).

Although neem has shown every indication of being safe to mammals in normal use, the possibility of future hazards should not be ignored (Anon, 1992; Raizada et al., 2001). Its residual persistence on foods is also unknown. Moreover, there is very little information on physiology (Jacobson, 1986) and toxicity of azadirachtin (Raizada et al., 2001). Furthermore, most toxicity studies on azadirachtin have been done on insects which show rapid loss of mobility and reduced fitness (Akudugu et al., 2001).

In view of a lack of or little information on its toxicity profile, an attempt has been made in the present study to evaluate the short-and long-term toxicity of azadirachtin of neemix-4.5 (a commercial botanical pesticide derived from the neem tree) in male and female of SWR/J mice.

2. Material and methods

Inbred normal SWR/J male and female mice, 8–10 weeks old and weighing 22.55–26.72 g, were used throughout the study. The animals were kept and bred in an environmentally controlled room with a temperature of 22 ± 1 °C, a relative humidity of 45 ± 5% and a light/dark cycle of 10/14 h. Mouse food (commercially available in Saudi Arabia) and water were offered *ad libitum*.

A total of 100 males and 100 females were used and were divided into 20 groups, 10 animals in each group. Groups 1–5 (males) and 6–10 (females) were orally treated once daily with the dose level 9.0 mg/kg body weight (1/10 LD_{50}) of azadirachtin of neemix-4.5 (Thermo Trilogy Corp., USA) dissolved in sterile distilled water for 2, 4, 6, 8 or 11.5 (80 days) weeks. Control mice (groups 11–15 and 16–20) were similarly treated with the corresponding volumes of the vehicle alone. At the end of each duration period, animals were weighed and then killed by cervical dislocation. They were then dissected and the weights of their livers, kidneys, spleens, hearts, lungs, testes or ovaries were recorded. Those organs from the 80-day treated groups and their controls were immediately fixed in Bouins fixative, processed for the usual paraffin embedding and 7.0 μm thick paraffin sections were cut according to the methods of Drury and Wallington (1967) and Humason (1979), stained with haematoxylin and eosin and then examined for histopathological changes. Moreover, the numbers of spermatozoa in the testes of males of control and azadirachtin of neemix-4.5-treated groups were determined (Bhunya and Behera, 1987).

The data obtained were analyzed statistically using the student's *t*-test and a 2×2 contingency table (X^2) (Sokal and Rohlf, 1981).

3. Results

Data in Table 1 show that azadirachtin of neemix-4.5 at the dose level 9.0 mg/kg body weight has significantly ($p < 0.05$)

Table 1 Effects of the dose level 9 mg/kg of azadirachtin of neemix-4.5 applied at different durations on the body weight and other parameters of treated SWR/J male mice.

Treatment duration	No. of males used	Body wt. in g at the start of exp. (Mean ± SE)	Body wt. in g at the end of exp. (Mean ± SE)	Liver wt. in g (Mean ± SE)	Kidney wt. in g (Mean ± SE)		Heart wt. in g (Mean ± SE)	Spleen wt. in g (Mean ± SE)	The two-lung wt. in g (Mean ± SE)	Testis wt. in g (Mean ± SE)		No. of sperm/ml (×10^6) (Mean ± SE)
					R-kidney	L-kidney				R-testis	L-testis	
Control	10	25.23 ± 0.44	32.67 ± 0.59	1.94 ± 0.11	0.29 ± 0.02	0.28 ± 0.02	0.18 ± 0.01	0.27 ± 0.03	0.25 ± 0.01	0.131 ± 0.003	0.131 ± 0.03	43.10 ± 2.04
2 weeks	10	24.32 ± 1.44	30.63 ± 1.44	2.03 ± 0.12	0.23 ± 0.02*	0.23 ± 0.02*	0.19 ± 0.01	0.24 ± 0.01	0.26 ± 0.01	0.126 ± 0.007	0.127 ± 0.002	39.21 ± 1.84
Control	10	24.43 ± 0.84	32.85 ± 0.63	2.14 ± 0.09	0.28 ± 0.01	0.28 ± 0.01	0.16 ± 0.01	0.25 ± 0.01	0.24 ± 0.01	0.128 ± 0.003	0.126 ± 0.003	38.00 ± 2.29
4 weeks	10	25.65 ± 0.86	31.15 ± 1.41	1.82 ± 0.05**	0.22 ± 0.01*	0.21 ± 0.01*	0.17 ± 0.01	0.23 ± 0.04	0.23 ± 0.01	0.118 ± 0.003*	0.117 ± 0.003*	29.67 ± 2.26*
Control	10	26.38 ± 0.14	33.98 ± 1.03	2.22 ± 0.09	0.30 ± 0.02	0.29 ± 0.02	0.17 ± 0.01	0.26 ± 0.03	0.25 ± 0.01	0.135 ± 0.009	0.132 ± 0.009	40.67 ± 1.46
6 weeks	10	25.64 ± 0.71	27.10 ± 1.29**	1.85 ± 0.13**	0.22 ± 0.02*	0.21 ± 0.02*	0.17 ± 0.01	0.21 ± 0.01*	0.25 ± 0.02	0.108 ± 0.009**	0.107 ± 0.009*	28.65 ± 1.47**
Control	10	26.51 ± 1.47	35.07 ± 2.03	2.25 ± 0.09	0.29 ± 0.02	0.29 ± 0.02	0.18 ± 0.01	0.24 ± 0.01	0.27 ± 0.01	0.141 ± 0.005	0.143 ± 0.003	39.89 ± 0.75
8 weeks	10	24.86 ± 0.46	26.86 ± 0.69**	1.74 ± 0.07**	0.20 ± 0.01**	0.20 ± 0.01**	0.15 ± 0.01*	0.14 ± 0.01*	0.25 ± 0.01	0.093 ± 0.003**	0.095 ± 0.002**	28.18 ± 1.08**
Control	10	26.72 ± 0.71	41.32 ± 1.22	2.34 ± 0.07	0.32 ± 0.01	0.31 ± 0.01	0.18 ± 0.01	0.26 ± 0.01	0.26 ± 0.01	0.136 ± 0.003	0.134 ± 0.005	42.10 ± 2.32
80 days	10	25.29 ± 0.38	32.63 ± 1.85**	1.86 ± 0.6**	0.25 ± 0.01**	0.25 ± 0.01**	0.15 ± 0.01*	0.16 ± 0.01*	0.26 ± 0.01	0.115 ± 0.005**	0.116 ± 0.004**	32.50 ± 2.36**

* Differences are statistically significant from the control at $p < 0.05$.

** Differences are statistically significant from the control at $p < 0.01$.