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Short communication

Mammalian safety of Decaleside II in the laboratory mouse



Y. Rajashekar*, T. Shivanandappa

Department of Food Protectants and Infestation Control, CSIR – Central Food Technological Research Institute, Council of Scientific and Industrial Research, Mysore 570 020, India

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ABSTRACT

Decaleside II, a novel trisaccharide isolated from the edible roots of *Decalepis hamiltonii*, belongs to a new class of natural insecticides. We have evaluated the mammalian safety of Decaleside II in the laboratory mouse. Our results on acute and sub acute toxicity study suggest that Decaleside II is not toxic to the laboratory mice as there were no symptoms of toxicity or mortality up to 2400 mg/kg bw. Haematological profile was unaltered and serum profiles of enzymes were not significantly affected. The lack of toxicity of Decaleside is attributed to the 1,4 α linkage of the sugars which are easily hydrolyzed by the digestive enzymes such as glucosidases. The selective toxicity to insects and mammalian safety of Decaleside II makes them highly suitable for use as novel grain protectants of natural origin.

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1. Introduction

Many insecticides have been replaced by modern insecticides due to environmental concerns and human health hazards [1,2]. Since the advent of agriculture by man, plants have been used for insect pest control and grain protection. Azadirachtin, a natural insecticide from the well known neem (*Azadirachta indica*) tree, is an antifeedant and insect growth regulator, but not commercially successful due to lack of contact toxicity, but finds use mainly in integrated pest management [3]. Rotenone, from the Derris root, one of the earlier plant-derived insecticides, was not acceptable (complete) because of its mammalian toxicity [4]. Widely used and successful synthetic pyrethroids were originally

derived from the flowers of *Tanacetum cinerariaefolium* [5]. Compounds with new mode of action are being discovered to deal with the problem of resistance and selectivity [6,7].

In view of the recent revival of interest in biopesticides, mammalian safety assessment of natural insecticidal compounds is of paramount importance [8]. Among the several biopesticides/bioactive compounds only a handful of them been tested for their toxicity and safety viz.: azadirachtin [9], pyrethrins [10], linalool, nictotine [11], ryania, Sabadilla [12], spinosad [13].

Recently we have reported a new class of natural insecticides from the edible roots of *Decalepis hamiltonii* named Decalesides [14]. Decalesides (I & II) are novel trisaccharides that are toxic to various insects species including stored product insects by contact exposure and exhibit a unique mode of action targeting gustatory sites in the tarsal sensilla. Being natural trisaccharides in their chemical nature they could serve as ideal candidates for a grain protectant in view of their eco-friendly nature and mammalian safety. In this study, we have investigated the acute and subacute toxicity of Decaleside II, insecticide compound, in the laboratory mouse.

Abbreviations: NADH, nicotinamide adenine dinucleotide – reduced; GOT, glutamic oxaloacetate transaminase; GPT, glutamate pyruvate transaminase; ALP, alkaline phosphatase; LDH, lactate dehydrogenase; NAD, nicotinamide; BSA, bovine serum albumin; EDTA, ethylenediamine tetraacetic acid; D, days; h, hours; bw, body weight; LD₅₀, lethal dose, 50%.

* Corresponding author. Tel.: +91 821 2513210; fax: +91 821 2517233.
E-mail address: rajacftri@yahoo.co.in (Y. Rajashekar).

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2. Materials and methods

2.1. Chemicals

Tris, L-alanine, lactate dehydrogenase, 2-oxoglutarate, nicotinamide adenine dinucleotide – reduced (NADH), α -ketoglutarate, bovine serum albumin, ammonium molybdate, ascorbic acid, and other chemicals were purchased from Sisco Research Laboratory, Mumbai, India. Commercially available enzyme assay kits for glutamic oxaloacetate transaminase (GOT), and Glutamate pyruvate transaminase (GPT) were purchased from Aspen Laboratory Pvt. Ltd. New Delhi, India. Drabkin's solutions, Cyanmethemoglobin were purchased from Span Diagnostics Ltd. Surat, India.

2.2. Animals

Adult swiss albino mice bred in our animal colony (38–42 g) were individually housed in polypropylene cages and provided with feed and water ad libitum.

2.3. Decaleside II

Decaleside II, the natural insecticide was isolated and characterised from methanolic extract of the roots of *D. hamiltonii*, as described earlier [14].

2.4. Mammalian toxicity

2.4.1. Acute toxicity

Animals were divided into five groups of four mice each. The animals were administered orally with a single dose of Decaleside II dissolved in water (200, 800, 1600 and 2400 mg/kg bw). The control group received water only. The animals were under observation for one week after the treatment.

2.4.2. Sub acute toxicity

Adult swiss albino mice were divided into three groups of six animals each. The animals were oral administered daily with Decaleside II at 50 and 500 mg/kg bw for 7 d to group II and III, respectively. The control group was administered with the water only. At the end of 7 d, the animals were sacrificed by ether anaesthesia and blood was collected in dry test tubes containing EDTA for haematological analysis.

2.5. Biochemical assays

2.5.1. Serum enzymes

Serum GOT and GPT were assayed as per manufacture's protocols originally based on Bergmeyer et al. [15] and Thomas [16], respectively. The serum alkaline phosphatase (ALP) was assayed using p-nitrophenyl phosphate as the substrate [17], serum lactate dehydrogenase (LDH) was assayed by UV method by the reduction of NAD with lactate as the substrate [18]. Protein content was measured by the method of Lowry et al. [19] using BSA as the standard.

2.6. Statistical analysis

The data was analysed using One-way Anova (analysis of variance) using the computer programme and Statplus 2007 software.

3. Results

3.1. Acute and sub acute toxicity

The treated animals were active, and appeared normal and showed no signs of toxicity. There was no toxicity (data not shown). Food intake was not affected. Body weight gains in the treated groups were comparable to the control group and were not significantly different. Organ weights of treated animals were also comparable to the control group (Table 1).

3.2. Haematology

Haemoglobin content, total erythrocyte count and leucocyte count, packed cell volume of the treated animals were not significantly different from those of the control group (Table 2).

3.3. Histopathology

Histological examination of the vital organs (liver, kidney, brain and testis) of treated mice did not show alterations indicative of toxicity. The histological profile was similar to that of control group (data not shown). The serum marker enzymes, GOT, GPT, ALP and LDH, in the treated groups, were not significantly altered when compared to the control group (Table 3).

4. Discussion

Global market trend towards grain protectants is increasingly concentrated on products derived from natural sources. As these biopesticides/plant extracts contain one or more chemical compounds, the safety evaluation becomes necessary in order to ensure safety to man. Many of the botanicals including natural grain protectants have not been thoroughly investigated for their mammalian toxicity. Some of the plant derived insecticides such as azadirachtin, nicotine, pyrethrin, rotenone, ryania, sabadilla, spinosads have been evaluated for the mammalian safety [1,4].

Natural pyrethrins are moderately toxic to mammals (acute oral LD₅₀ 1200 mg/kg bw to rat), but technical grade pyrethrum was reported to be less toxic [10]. Nicotine is highly toxic to mammals (rat oral LD₅₀ = 50 mg/kg bw), and hazardous to human health, and, therefore, banned in many countries [20]. Rotenone is highly toxic to mammals (rat oral LD₅₀ = 134 mg/kg bw) [4]. Spinosad, a recently introduced insecticide of natural origin, shows very low toxicity to mammals with acute oral LD₅₀ of 3738 mg/kg bw to male rats [21]. Mammalian toxicity of other natural insecticides is variable (Table 4).

In this study, we have evaluated Decaleside II for the mammalian safety. Our results on acute and sub acute

Table 1

Sub acute (multiple doses) toxicity study of oral administered of Decaleside II to male mice: organ weights.

Group	Relative weight (g/100 g bw)			
	Liver	Kidney (mean ± SD)	Brain	Testis
Control	1.92 ± 0.2	0.288 ± 0.04	0.501 ± 0.05	0.147 ± 0.03
Decaleside II treated 50 mg/kg bw	1.98 ± 0.3	0.289 ± 0.08	0.496 ± 0.05	0.145 ± 0.01
Decaleside II treated 500 mg/kg bw	1.89 ± 0.32	0.284 ± 0.03	0.483 ± 0.04	0.154 ± 0.02

The values were mean ± S.E. (n = 10) in each group.

There was no significant different between control and treatment group in the same sex (n = 4, error bars, s.e.m.), One-way ANOVA, P < 0.001.

Table 2

Sub acute toxicity (multiple doses) study of Decaleside II to mice: haematological profile.

Parameter	Control	Decaleside II treated (50 mg/kg bw) (Mean ± SD)	Decaleside II treated (500 mg/kg bw)
Haemoglobin (g/dl)	13.9 ± 2.1	14.5 ± 2.4	14.3 ± 1.9
RBC (10 ⁶ /ml)	10.6 ± 0.8	10.3 ± 1.0	9.9 ± 0.6
WBC (10 ³ /ml)	7283 ± 212	7800 ± 220	8250 ± 156
PCV (%)	39.3 ± 3.5	40.3 ± 2.1	38.3 ± 3.2

The values were mean ± S.E. (n = 10) in each group.

There was no significant different between control and treatment group in the same sex (n = 4, error bars, s.e.m.), One-way ANOVA, P < 0.001.

Table 3

Sub acute toxicity of Decaleside II to mice: serum enzymes.

Serum biochemical parameter (units/L)	Control	Decaleside II treated (50 mg/kg bw) (Mean ± SD)	Decaleside II treated (500 mg/kg bw)
GOT (U/L)	26.7 ± 1.8	24.6 ± 2.2	26.1 ± 1.9
GPT (U/L)	16.6 ± 2.1	14.3 ± 2.6	15.2 ± 2.4
LDH (U/L)	60.3 ± 2.1	61.1 ± 2.0	61.8 ± 3.2
ALP (U/L)	42.3 ± 4.8	44.6 ± 3.3	45.1 ± 3.1

Glutamate oxaloacetate transaminase (GOT); glutamate pyruvate transaminase (GPT); lactate dehydrogenase (LDH); alkaline phosphate (ALP). Each bar represents the value of mean ± SE, n = 6. There was no significant different between control and treatment groups in the same (n = 4, error bars, s.e.m.), One-way ANOVA, P < 0.001.

toxicity study suggest that Decaleside II is not toxic to the laboratory mouse. Decaleside II was not toxic up to 2400 mg/kg bw in acute toxicity study. In the sub acute study the Decaleside II did not show any toxicity up to 500 mg/kg bw when orally administered for 7 days. There were no histopathological alterations in the vital organs

Table 4

The mammalian toxicity of some natural insecticides [23].

Natural insecticides	Mammalian toxicity Oral rat LD ₅₀ (mg/kg bw)
Anethole	2090
β-Asarone	275
Azadirachtin	13,000
Carvacrol	810
Cineole (1–8)	2480
Cinnamaldehyde	1160
Cuminic aldehyde	1390
Eugenol	500
Nicotine	50
Pyrethrin I and II	1200
Rotenone	350
Ryania	750
Sabadilla	5000
Spinosad	3738

including the liver. Haematological profile was unaltered and comparable to that of control mice. Serum profile of enzymes such as GOT, GPT, ALP and LDH was not significantly changed indicating lack of hepatic damage.

The tuberous roots of *D. hamiltonii* have been consumed by man for centuries, and there is no report of adverse effects on human health. Earlier studies of the roots extracts of *D. hamiltonii* have shown no mammalian toxicity in rats in a 90 d study [22]. Decaleside II, present in the roots of *D. hamiltonii* appears to be safe for man. Since availability of the purified compound is limited, long-term studies were not done. The lack of toxicity of Decalesides is attributable to the 1,4 α linkage of the sugars which are easily hydrolyzed by the digestive enzymes such as glucosidases [14]. Starch and glycogen and other carbohydrates present in food, also contain 1,4 α linkage which are broken down by the enzymes in the body, and therefore, readily digested. The insect selectivity and mammalian safety of Decalesides, as shown by our studies, makes them highly suitable for use as novel grain or seed protectants of natural origin.

Conflict of interest

Authors have declare that no conflict of interest.

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