

Detection of castor allergens in castor wax

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Summary

The presence of castor bean allergens in castor wax products was determined by *in vivo* and *in vitro* analysis of castor wax extracts. Allergens were detected in one extract of castor wax by the PCA reaction in mice, the RAST inhibition reaction, and skin prick test in castor bean sensitive individuals. However, these allergens in the wax were of much lower potency than those in the bean, and were not detectable in a deodorant product utilizing castor wax.

Introduction

Castor plants (*Ricinus communis*) are cultivated commercially in subtropical countries. Castor beans yield and oil which has many commercial uses due to its high viscosity, resistance to heat and pressure, low freezing point and ability to form a waxy substance upon chemical treatment. After extraction of oil from the bean, the dry powdery residue, castor pomace, remaining is used as a fertilizer or as an animal feed. Layton and co-workers and Panzani have demonstrated that castor bean and castor bean products contain potent allergens capable of inducing attacks of asthma accompanied by urticaria (Layton, Lee & De Eds, 1961; Layton *et al.*, 1962; Panzani, 1957; Panzani & Layton, 1963). Initial investigations suggest that castor pomace but not castor oil is allergenic (Panzani & Layton, 1963). However, this issue has not been completely resolved because an allergic reaction has been reported to polyoxyethylated castor oil (Mehta, 1973). Owing to the extensive use of castor oil and wax in industry and medicine, this issue is of growing concern. The following study was directed toward determining if castor bean allergens are present in castor wax products.

Materials and methods

Extraction of castor beans

Aqueous extracts of castor beans were prepared by homogenization of beans with

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phosphate (0.01 M) buffered (pH 7.2) saline (0.15 NaCl) (PBS) in a waring blender as described (Lehrer, Karr & Salvaggio, 1977, 1978). Extracts were heated at 100°C for 1 hr to destroy ricin, a castor bean toxin, centrifuged (20,000 g), concentrated (Amicon UM10) and recentrifuged (105,000 g). The dry weight concentration was determined gravimetrically and protein concentration by the Folin reaction (Lowry *et al.*, 1951). The presence these extracts of castor bean allergens has been shown by the induction of reaginic antibody production in mice (Lehrer *et al.*, 1977, 1978), the radioallergosorbent test (RAST) assay, and skin tests of sensitive individuals (Karr *et al.*, 1978).

Extraction of castor wax

Aqueous extracts of castor wax were prepared using the following solvents—PBS, 4 M urea—1 M NaCl, 0.1% triton X-100, or 0.1% sodium lauryl sulphate (SLS). For each extraction, 200 g of castor wax were homogenized in approximately 1.5 l extraction solvent for 1 min, medium speed (Waring GB-1 commercial blender). Following homogenization, solvent was added to the homogenate to a volume of 2 l and the homogenate mixed at 24°C overnight. Extracts were filtered through 2 layers of gauze and then through Whatman No. 1 filter paper. The filtrates were concentrated (UM10 Amicon filter), dialyzed against their own extraction solvent, and centrifuged (105,000 g). Supernates were removed, snap frozen and stored at -20°C until used. The dry weight concentration was determined gravimetrically or by organic matter determination (Marsh & Weinstein, 1957).

Castor wax was also solubilized by heating in the following solvents: 400 mg in 10 ml of Fluid AP; 1200 mg in 10 ml isopropylpalmitate; 1,000 mg in 10 ml ethanol; 1,000 mg in 10 ml isopropanol. Upon cooling to 24°C, the wax solution formed a semi-solid material which was used in further tests.

Qualitative precipitation analysis

Aqueous extracts of castor wax were analyzed for castor bean antigens with rabbit antiserum by immunodiffusion in gel utilizing 1% agarose in PBS. Each well contained 50 µl antigen or antiserum. The antiserum was produced in rabbits immunized with extracts of castor bean in Freund's complete adjuvant as described (Lehrer *et al.*, 1977).

Mouse reaginic antibody analysis

Aqueous extracts of castor beans and castor wax were analyzed by the passive cutaneous anaphylaxis (PCA) reaction in mice. It is well documented that the 48 h PCA reaction will detect reaginic (IgE) antibodies (Lehrer 1976, 1977). Recipient CFW female mice (Charles River Laboratories) were sensitized with reaginic antibody (produced in BDF mice immunized with heated castor bean extracts and *B. pertussis* adjuvant as described, Lehrer *et al.*, 1977). These animals were challenged 48 h later with an intravenous injection of extracts of castor beans or wax in 0.5% Evans blue solution, and killed 30 min later. A positive response was a blue spot ≥ 5 mm on the underside of the skin. Controls consisting of non-sensitized mice challenged with castor wax extracts were always negative.

Radioallergosorbent test (RAST) inhibition assay

A highly sensitive assay for IgE antibodies to castor bean allergens, developed utilizing the RAST technique, was adopted to detect allergens in unknown samples (Gleich *et*

al., 1974). One hundred μ l of serum and 100 μ l of castor bean, wax extract, or PBS were incubated with antigen coated discs on a rotator overnight at 24°C. This was followed by washing, the addition of radioiodinated anti-IgE, repeated incubation, washing and counting in a Beckman Bio Gamma counter. Controls consisted of normal serum incubated with antigen coated discs and test serum incubated with discs coated with a non-cross-reacting antigen. Results were expressed as the percentage binding radioiodinated anti-IgE as compared to patient serum preincubated with PBS.

Skin tests of sensitized individuals

In order to assess clinical sensitivity to castor wax extracts and products, skin tests were performed on individuals with known castor bean sensitivity (as determined by RAST, skin reactivity to castor allergen and clinical history (Karr *et al.*, 1978). This test detects IgE antibody specific for castor bean allergen. The castor bean or wax extracts were applied to the skin using both patch and prick test techniques. Patch tests, useful in the detection of cell mediated hypersensitivity, were performed by applying gauze containing castor wax or paraffin (control) against the skin for 24 and 48 h. The skin reaction was read at 24 and 48 h after removal of the patch. A positive patch test consists of induration and vesiculation compared to negative controls. Prick testing, which is useful for detecting IgE mediated hypersensitivity, was performed by applying a drop of allergen extract to the skin and then 'pricking' the underlying superficial layer of skin with a 25 gauge needle. Prick tests were read at 15 min following antigen application. A positive prick test consists of a weal and flare reaction with oedema > 5 mm.

Results

Castor extracts

The yields of the different extractions of castor wax are summarized in Table 1. PBS and urea-NaCl solvents were not very effective and solubilized only small amounts of the castor wax. SLS was more affective and extracted 0.84% of the material, whereas triton X-100 extracted the greatest amount, 1.52% of the castor wax.

All of the extracts were analyzed for total organic matter and protein. Protein was expressed as a percentage of the total organic matter or dry weight and results are summarized in Table 2. Castor bean extract served as a standard for the total organic matter determination, thus the total organic matter equalled the dry weight determination. The Folin assay demonstrated that a considerable amount (60%) of castor bean extract was protein. Extracts of castor wax utilizing PBS or urea-NaCl were ineffective and dry weight determinations did not detect any material solubilized. The organic matter determination, a more sensitive method, did detect a small amount of organic

Table 1. Yield of castor wax extracts

Extraction solvent	Castor wax solubilized (%)
PBS	0.0013
4M Urea + 1 M NaCl	0.00046
Triton X-100	1.52
SLS	0.84

Table 2. Dry weight, organic matter and protein concentrations of castor wax extract

Extract	Dry weight (mg/ml)	Organic matter* (mg/ml)	Protein†	
			mg/ml	percentage
Castor bean	62.3	62.3	37.4	60.0
Castor wax-PBS	<0.1	0.15	0.02	12.9
Castor wax-urea/NaCl	<0.1	0.05	0.15	100.0
Castor wax-triton	150.0	145.0	0.5	0.34
Castor wax-SLS	61.0	44.0	10.4	17.0

* Method of Marsh & Weinstein, 1966, castor bean extract used as standard.

† Folin reaction, BGG used as standard.

material present in these extracts. When protein was assayed in these extracts, it was shown that 12.9% of the organic matter in the PBS extract was protein, whereas all of it was protein in the urea-NaCl extract. The greatest amount of material extracted was by the triton X-100 and SLS solvents. Although the triton X-100 solvent yielded the greatest amount of material solubilized, less than 1% of this was protein. However, the SLS extract contained 17% protein.

Detection of castor allergens and antigens

All extracts were analyzed for castor antigens by reactivity of rabbit antisera to castor extract. These antisera had been shown to detect several castor antigens by immunoprecipitation in gel (Lehrer *et al.*, 1978). Of the castor extracts tested, only the SLS wax extract reacted with this antisera. SLS solution alone, had no reactivity with the antiserum. However, this reaction was unusually strong suggesting a false-positive reaction. Therefore, this substance was tested with normal rabbit serum and the same precipitin line was demonstrated (Fig. 1). It was therefore concluded that this was probably a non-specific reaction of SLS castor wax extract with rabbit serum proteins.

These extracts were also analyzed for allergens by reactivity with mouse anti-castor reaginic antibody as described. As shown in Table 3, this antiserum demonstrated a PCA titre of 844 to castor bean extract. In spite of its high reactivity with castor bean extract, this antiserum did not react with PBS, urea-NaCl, or triton X-100 extracts of castor as shown by the negative PCA reaction. However, a positive response was detected with this antiserum to SLS castor wax extract as shown by the positive PCA titre of 40 in the bottom portion of Table 3.

Further analysis of allergens in castor wax extracts was made by the RAST inhibition assay. Results are summarized as percentage inhibition of the patients RAST (Table 4). Although this assay could detect up to 7.5 µg castor allergen, no inhibition was detected with PBS or triton X-100 extracts and only a small inhibition demonstrated with urea-NaCl extract. However, a significant inhibition of the castor RAST reaction was demonstrated when SLS castor wax extract was used. Since it was known that this extract of castor wax can non-specifically react with proteins (as shown by the precipitation with normal rabbit serum), it is possible that it may inhibit the RAST non-specifically and therefore it was tested using a heterologous RAST inhibi-

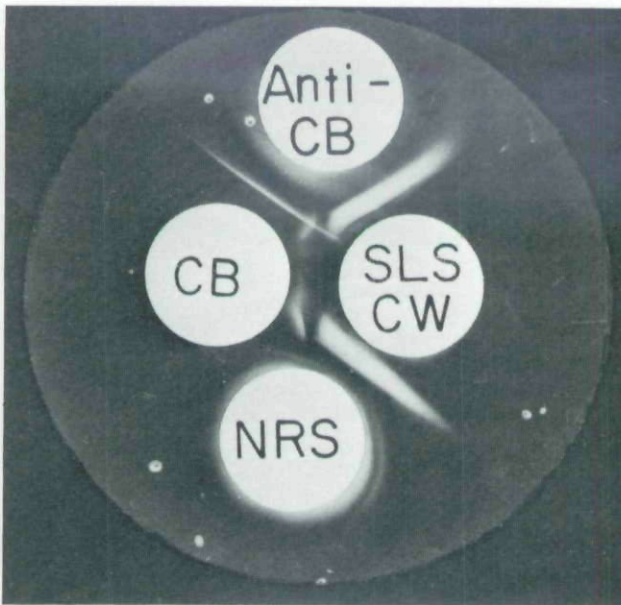


Fig. 1. Reactivity of immune or normal rabbit serum with SLS castor wax extract. Each well contains 50 μ l of rabbit serum or castor SLS wax extract. Plates were incubated at 24°C for 48 hr, and then washed exhaustively with 0.15 M citrate in PBS to reduce non-specific precipitation.

Table 3. Reaction of castor wax extracts with mouse anti-castor antigen

Antiserum	Challenge antigen	PCA* Titre
Anti-castor	200 μ g castor bean	844
Anti-castor	30 μ g PBS-castor wax	< 5
Anti-castor	200 μ g urea NaCl-castor wax	< 5
Anti-castor	290 μ g triton-castor wax	< 5
Anti-castor	890 μ g SLS-castor wax	40

* Mean titre of 3 determinations.

tion assay. Extracts of castor bean, SLS castor wax or triton castor wax extract were incubated with reaginic antisera from a patient sensitive to peanuts and then assayed in the peanut RAST. Results are expressed as percentage inhibition in Table 5. As shown in this Table, castor bean extract did not inhibit the peanut RAST reaction indicating that castor and peanut allergens are immunochemically distinct. However, the SLS castor wax extract inhibited the peanut RAST by 37%. This suggests that the results in Table 4 may be due entirely or in part to a non-specific inhibition of the RAST reaction

Table 4. Detection of castor bean allergen in castor wax extracts by RAST inhibition assay

Sample tested	Amount (μ g)	RAST inhibition (%)
Castor bean	750	84.0
Castor bean	75	27.0
Castor bean	7.5	10.3
Castor bean	0.75	0.0
Castor wax-PBS	35	0
Castor wax-urea-NaCl	6200	13.4
Castor wax-triton X-100	1450	0
Castor wax-SLS	4450	40.1

Table 5. Inhibition of peanut RAST by castor wax extracts

Extract	Amount (mg)	RAST inhibition (%)
Castor bean	6.2	0
SLS-Castor wax	4.45	37
Triton-Castor wax	1.45	6

rather than indicating that the SLS castor wax extract contains specific castor allergens.

In order to determine whether the SLS castor wax extract contains specific allergens or merely non-specifically inhibits the RAST assay, the following direct RAST study was done. Discs were coated with castor bean or the SLS castor wax extract (Lowry *et al.*, 1951) and a direct RAST assay done with serum from a patient sensitive to castor allergens (JR), a patient sensitive to peanuts (SJ), or pooled human serum (PHS) used as a control. Results, expressed as a RAST ratio of experimental values to those obtained with the PHS, are summarized in Table 6. As shown, JR had a

Table 6. Detection of castor allergens by RAST

Serum	Antigens	cpm	RAST ratio*
JR	Castor bean	17781	18.87
	SLS-Castor wax	13975	8.04
SJ	Castor bean	902	0.96
	SLS-Castor wax	3450	1.96
PHS	Castor bean	945	—
	SLS-Castor wax	1738	—

* RAST ratio = $\text{ct/min JR} \div \text{ct/min SJ} \div \text{ct/min PHS}$

significant RAST ratio of 18.8 to castor bean allergen and a significant ratio of SLS castor was of 8.0. However, SJ did not have a significant RAST to either castor bean or castor wax extracts. These results indicate that although SLS castor wax extract can non-specifically inhibit the RAST assay as shown in Tables 4 and 5, this extract does contain castor bean allergens as shown by the direct RAST reaction in Table 6.

Castor allergen skin tests

Seven normal laboratory personnel (VA, DL, CO, FC, HB, DW, and MM) were skin tested with increasing concentrations of castor bean or SLS castor wax extract. The results, summarized in Table 7, demonstrate that no immediate skin reactivity was

Table 7. Immediate skin reactivity of castor wax extracts in normal and sensitive individuals

Subject	Positive prick test reaction	
	Castor bean*	Castor wax*
VA	> 1,000	> 22,000
DL	> 1,000	> 22,000
CO	> 1,000	> 22,000
FC	> 1,000	> 22,000
HB	> 1,000	> 22,000
DW	> 1,000	> 22,000
MM	> 1,000	> 22,000
LE	0.1	10,000
LT	0.1	1,000
SS	1.0	100

* Minimal concentration in $\mu\text{g/ml}$ eliciting a wheal 3 mm in size greater than saline control.

demonstrated with either castor bean extract (up to a concentration of 1 mg/ml), or castor wax extract (up to a concentration of 22 mg/ml). These extracts were then used in skin tests of three individuals who had developed occupational hypersensitivity to castor allergens (LE, LT and SS). The results demonstrate that these individuals were highly sensitive to castor allergen as shown by the positive skin reaction to 1.0 or 0.1 $\mu\text{g/ml}$ castor bean extract. These individuals also demonstrated a positive skin reaction to SLS castor wax extract, although the level of sensitivity was 100 to 100,000 fold less than that using castor bean extract.

Castor wax or paraffin were tested for delayed skin reactivity by the patch test as described earlier. Results demonstrated that none of the normal or castor sensitive subjects demonstrated delayed skin reactivity to any of the castor wax extracts in spite of the fact that one subject, SS, demonstrated a delayed response to castor bean extract.

Since immediate hypersensitivity to castor wax extract was demonstrated in castor bean sensitive subjects, further tests were done to determine if castor wax solubilized in different organic solvents or castor wax present in a deodorant stick would irritate and elicit a positive immediate hypersensitivity reaction in these individuals as determined by the prick test method. No positive skin reactions were detected to any of these extracts in castor bean sensitive individuals.

Discussion

Castor beans are an important source of oil and wax for industry. In spite of the evidence that castor oil does not contain the potent castor bean allergen (Panzani *et al.*, 1963), concern exists because a hypersensitive reaction to polyoxyethylated castor oil has been reported and castor wax is used in different industrial and medical products. The present study used different methods of extracting castor wax and these extracts were tested by a variety of procedures in order to detect allergens. Results demonstrate that allergens are present at low concentrations in castor wax since the SLS wax extract produces positive PCA reactions in mice, a positive direct RAST for reaginic castor allergens and yields positive skin reactions in sensitized individuals. No delayed response was detected to castor wax in these subjects although one subject (SS) gave a delayed response to castor bean extract.

The demonstration of castor allergen in wax is of concern since wax is extensively used in a variety of industrial and medical products. Our detection of allergen supports the earlier observation of a reactivity to polyoxyethylated castor oil which is used as a solvent for althesin (Mehta, 1973). These results suggest that a survey of several batches of castor oil and castor wax should be done using *in vitro* methods to see if the presence of the allergen might become a problem under extreme conditions.

Based on our *in vitro* results, preliminary tests were made with the underarm antiperspirant and deodorant product to see if a positive reaction could be detected by the prick test in castor bean sensitive individuals. In spite of the fact that these individuals are exquisitely sensitive to castor bean extract and demonstrate positive skin reactivity to extracts of castor wax, no skin reaction was demonstrated by the prick test to the underarm deodorant product.

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