Detoxification of Castor Seed Meal by Interaction with Sal Seed Meal

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Castor (*Ricinus communis*) seed meal was detoxified by a novel method of wet mixing with sal (*Shorea robusta*) seed meal so that the toxic constituents of castor seed meal were neutralized by tannins, the toxicants present in the sal seed meal. The resulting product was innocuous, as revealed in the feeding studies in rats. The nutritional benefit of the treated material is improved by synergistic action of a protein such as casein. The aqueous extract of castor seed meal produced a smooth-muscle stimulant effect, whereas this effect was not observed with the extract from treated meal. This is a new concept of neutralization of two toxins by each other in two seed meals. The method can be employed to investigate the suitability of such a processed seed meal as an animal feedstuff ingredient.

KEY WORDS: Allergen, castor seed meal, detoxification, ricin, ricinine, sal seed meal, tannin.

Currently, India is the largest producer of castor seed (*Ricinus communis*) in the world. The whole seeds yield about 40% oil, which is extensively used in viscous lubricants, production of important oleochemicals, surface coatings, soaps, cosmetics and pharmaceuticals. The deoiled meal consists of about 25% protein and cannot be used as protein supplement for animal or human consumption due to the presence of the extremely toxic lectin called ricin, the much less toxic alkaloid ricinine and several allergens (1). The shortage of protein sources in India has necessitated a search for alternative sources. Beneficiation of castor seed meal for use in animal feedstuffs, instead of its current use as manure, would be of national economic importance. The potential availability of castor oilseed in India is about 470,000–500,000 tons per year. Ricin comprises about 1.5% of the meal and can agglutinate red cells (2). It is a glycoprotein with A and B polypeptide chains linked by a disulfide bond. The A chain inactivates the eukaryotic ribosome and inhibits protein biosynthesis by enzymatic action. The B chain contains galactose-binding sites. Ricin causes mortality at low doses (10 μg/kg) in laboratory animals. Seeds are poisonous to humans, horses, cattle, sheep, pigs and poultry (3). Ricinine is a relatively harmless alkaloid, amounting to 0.3% of the meal, and is insecticidal (4). Allergenic fractions, which are protein polysaccharides (CB-1A) ranging from 0.1 to 4%, were also isolated from the meal and can cause allergic manifestations upon ingestion or after inhalation of the powder (5). Several methods have been devised to detoxify the cake by wet and dry heating or autoclaving; by washing with water, alcohol and aqueous HCl; and treatment with formaldehyde and lime (6), but these methods are possibly not commercially feasible because the growth and feed conversion in rats fed these materials were lower than in the control (6). The reactions that are drastic enough to destroy castor toxins also destroy the useful proteins.

The ability of proteins to bind with tannins in solutions is well documented (7). We have effected the detoxification of castor seed meal by wet mixing with sal (*Shorea robusta*) seed meal, another tannin-containing seed meal abundantly available in India. Naturally-occurring food tannins react with proteins, including enzymes, to form tannin protein complexes that lead to inactivation of digestive enzymes. In this way tannins interfere with protein digestibility and absorption. The detoxification of castor seed meal by neutralization of its toxicants by tannins present in the sal seed meal and the biological studies to evaluate the toxicity and nutritive value of such processed material are described here.

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MATERIALS AND METHODS

The deoiled seed meals of castor and sal were obtained locally. The decorticated sal seeds were crushed and pelletized after steaming. The pellets were extracted with hexane to remove fat, and residual solvent was removed by steaming. The material was powdered in a hammer mill. Whole seeds of castor were pressed in an expeller to produce a cake with 6% oil. The residual oil was extracted with hexane to reduce the oil content to about 1%. The residual solvent was removed by steaming. The proximate compositions of the materials were determined according to Association of Official Analytical Chemists' methods (8) and are shown in Table 1. The protein levels in sal seed meal and castor seed meal were 11.7 and 27.1%, respectively. Fat was estimated by Soxhlet extraction with petroleum ether. Tannins were estimated by the Folin-Denis method (8). Crude fiber was estimated as loss on ignition of dried residue after 1.25% acid and alkali digestion (8). The reducing sugars and starch were estimated according to the procedure described by Friedemann *et al.* (9).

The treatment consists of wet-mixing 500 g of each of the two materials with 1 L of water in a Hobart mixer (The Hobart Manufacturing Company, London, England) for 15 min. Subsequently, the materials were ammoniated by mixing with 100 mL of 25% ammonia solution (liquor ammonia) for 30 min. The material was transferred to flat trays and dried at 60–70°C for 6–7 h and powdered in a hammer mill. The material is referred to as Tx-11. The ammoniation increased the nitrogen content by 0.75%. The proximate composition of this material (Table 1) was estimated according to methods described previously in this section. Electrophoresis of the proteins was carried

TABLE 1

Proximate Composition of Sal, Castor Seed Meals and Tx-11 (percentage)

	Sal seed meal	Castor seed meal	Tx-11
Moisture	7.3	8.1	8.3
Protein	11.7	27.1	19.7
Crude fiber	13.5	41.1	25.2
Reducing sugars	4.9	4.3	4.5
Starch	43.6	9.4	28.3
Ash	5.0	7.5	5.8
Fat	2.7	0.3	1.2
Tannins	9.0	0.5	4.5

out on polyacrylamide disc gel (7% acrylamide, Tris-glycine buffer pH 8.3) with aqueous extracts according to the procedure described by Davis (10). Raw castor seed meal (2 g) or 4 g of Tx-11 in 10 mL distilled water was warmed in a shaker waterbath for 30 min at 50°C and centrifuged. The supernatant was used for separation on polyacrylamide gel electrophoresis.

Effect on isolated rat fundus and guinea pig ileum. The aqueous extracts of castor seed meal or Tx-11 were tested for pharmacological activity on smooth muscle. Raw castor seed meal (1 g) or 2 g of Tx-11 in 10 mL distilled water was warmed in a shaker waterbath at 50°C for 30 min and centrifuged. The supernatant of each of the extracts (0.5 and 1 mL) were tested for any contraction on isolated rat fundus strip and guinea pig ileum mounted in a 10-mL Dale's organ bath according to a previously described procedure (11).

Feeding studies with Tx-11 at 20% in rats. This experiment was carried out to study the relative toxicity of untreated and treated materials in diets supplemented with casein. The control diet contained 15% casein, 6% groundnut oil, 4% Jones Foster salt mixture (12), 6% cellulose, 59% starch and 10% starch mixed with vitamins. The composition of the vitamins is shown in Table 2 (13). The incorporation of raw castor seed meal or Tx-11 was achieved by replacement of starch from the control diet. Haffkine Wistar rats were distributed into four groups of six each (three male + three female). The different diets contained the following levels of raw castor seed meal or Tx-11: Gr I, control; Gr II, raw castor seed meal at 10%; Gr III, raw castor seed meal at 20%; and Gr IV, Tx-11 at 20%.

The animals were housed individually. Food consumption and body weights were noted daily. The animals were sacrificed at the end of 92 d and organs were weighed. A histopathological examination of the organs was carried out.

Feeding studies with Tx-11 at 80, 78 and 76% in diets containing 0, 2 and 4% casein. This experiment was carried out to assess the toxicity of Tx-11 by feeding it at high levels and to assess whether the animals receiving Tx-11 can sustain growth without casein or with low levels of casein in diets. Haffkine wistar rats were distributed into six groups of four each (two male + two female) and were fed the following diets: Gr I, control at 0% casein, 80% starch; Gr II, control at 2% casein, 78% starch; Gr III, control at 4% casein, 76% starch; Gr IV, experimental at 0% casein, 80% Tx-11; Gr V, experimental at 2% casein, 78% Tx-11; Gr VI, experimental at 4% casein, 76% Tx-11.

The composition of the diets is shown in Table 2. Food consumption and body weights were noted daily. Mortality of the animals was noted, and the surviving animals were sacrificed at the end of seven weeks. Histopathological examination of the organs was carried out.

RESULTS

Polyacrylamide gel electrophoresis. The aqueous extract of raw castor seed meal showed seven dark bands on staining with amido black (Fig. 1A), whereas the extract of Tx-11 showed no bands (Fig. 1B). Soluble proteins, which include ricin and allergens, appeared as dark bands.

Isolated tissue experiments. Figure 2 depicts contractions produced with extracts of castor seed meal and Tx-11 on isolated rat fundus (upper panel) and guinea pig ileum

TABLE 2

Composition of the Diets (w/w%)[a]

	Groups					
	I	II	III	IV	V	VI
Casein	0	2	4	0	2	4
Starch + vitamins[b]	80	78	76	10	10	10
Salt mixture[c]	4	4	4	4	4	4
Groundnut oil[d]	6	6	6	6	6	6
Cellulose	10	10	10	0	0	0
Tx-11	0	0	0	80	78	76

[a]Rats were fed Tx-11 at 80, 78 and 76% for seven weeks.
[b]Thiamine HCl, 0.5 mg; riboflavin, 0.6 mg; pyridoxine HCl, 0.3 mg; pantothenic acid, 2.7 mg; nicotinic acid, 54 mg; choline chloride, 368 mg; biotin, 20 μg; vitamin B_{12}, 3 μg; inositol, 22 mg; folic acid, 1.5 mg; PABA, 10 mg; cystine, 15 mg; and ascorbic acid, 0.5 mg were added in 10 g starch and incorporated in diets of all groups.
[c]Jones Foster salt mixture (Ref. 11).
[d]325 I.U. of vitamin A acetate, 85 I.U. of vitamin D_2 and 10 mg DL-α-tocopheryl acetate were dissolved in 6 g of oil.

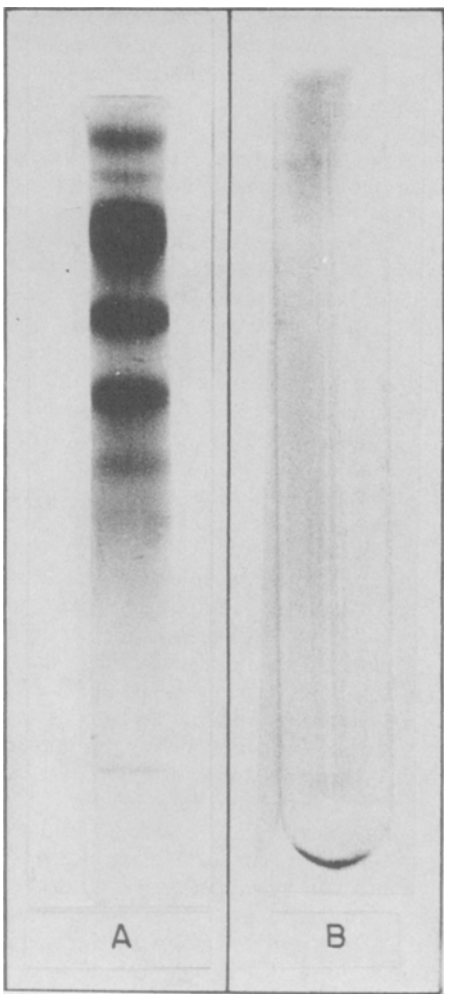

FIG. 1. Polyacrylamide gel electrophoresis of (A) extract of raw castor seed meal, showing seven bands, and (B) extract of treated meal, showing no bands.

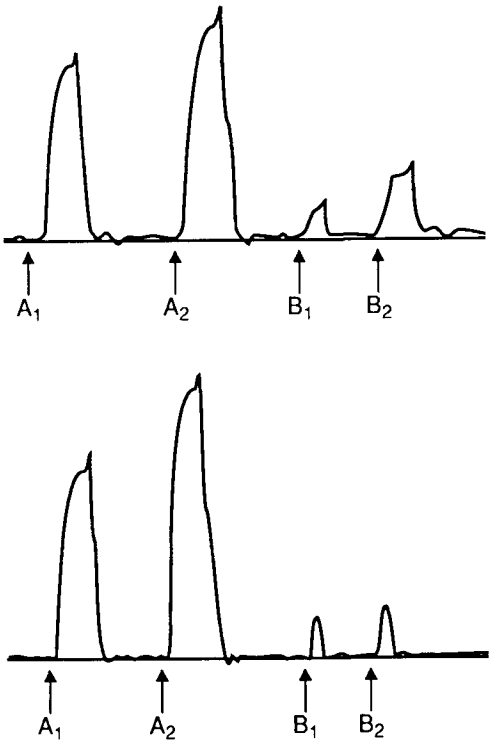

FIG. 2. Upper panel, isolated rat fundus; lower panel, isolated guinea pig ileum. A_1 and A_2, contractions due to aqueous extract from castor seed meal; B_1 and B_2, contractions due to aqueous extract from treated meal.

(lower panel). The aqueous extract of raw castor seed meal showed a dose-related contraction on isolated rat fundus as well as on guinea pig ileum (Fig. 2, A_1 and A_2), whereas the extract of Tx-11 showed almost no contraction (Fig. 2, B_1 and B_2).

Feeding studies with Tx-11 at 20%. All the animals fed raw castor seed meal at 20% died within a period of nine days. One of the animals fed raw castor seed meal at 10% died at the end of nine days. There was no mortality in the animals fed Tx-11 at 20% in the diet. The weight gain, food consumption and food efficiency ratio of the animals fed Tx-11 are shown in Table 3 and are almost comparable to those of control animals. The animals fed raw castor seed meal at 10% showed an 18 and 17% reduction in weight gain and food consumption, respectively (Table 3). The organ weights of liver and kidney of animals fed raw castor seed meal at 10% were significantly higher than those of the control ($P < 0.05$). The animals fed Tx-11 did not show any significant difference in organ weights as compared to those in the control (Table 4). The animals fed raw castor seed meal showed changes in the liver, such as disturbed hepatic cord and cloudy swelling. The animals fed Tx-11 did not show any such histological changes in the hepatic tissues. Other tissues did not show any abnormalities in any of the groups.

Feeding studies with Tx-11 at 80, 78 and 76%. All the animals fed the control diet with 80% starch (0% casein) died during the fourth week of the experiment. Three out of four animals fed the control diet containing 78% starch plus 2% casein died during the fifth week. There was no mortality in the animals fed the control diet containing 76% starch plus 4% casein, or in any of the experimental groups fed Tx-11 at 80, 78 and 76% with 0, 2 or 4% casein. The feed intake, weight gain and feed conversion of the rats fed Tx-11 in the three groups were significantly better than in the corresponding animals from control groups fed equivalent levels of dietary casein (Table 5). The growth of the rats fed diets containing 4% casein was much better in the groups fed Tx-11 than in the control (Fig. 3). At the end of the experiment, although there were no surviving animals in the control group (0% casein in diet), all the animals in the corresponding experimental

TABLE 3

Mortality, Body Weight, Food Consumption and Food Conversion Ratio at the End of the 92-Day Feeding Study

Group	Mortality	Initial weight (g)	Final weight (g)	Weight gain (g)	Food consumption (g)	Food efficiency ratio[a]
Control[b]	Nil	32 ± 3	233 ± 23	201 ± 24	1176 ± 64	0.171 ± 0.012
Tx-11 (20%)[b]	Nil	33 ± 3	231 ± 20	198 ± 20	1160 ± 62	0.171 ± 0.009
Raw castor seed meal (10%)[c]	1/6	33 ± 3	203 ± 32	170 ± 34	1031 ± 92	0.165 ± 0.018
Raw castor seed meal (20%)	6/6	33 ± 3	—	—	—	—

[a]Weight increase per gram of food consumed.
[b]Mean of six animals ± SE.
[c]Mean of five animals ± SE.

TABLE 4

Mean Relative Organ Weights (g/100 g body weight ± SE) of Rats Fed Tx-11 and Raw Castor Seed Meal for 92 Days

Group	Liver	Kidney	Heart	Spleen
Control[a]	3.290 ± 0.091	0.595 ± 0.019	0.303 ± 0.009	0.203 ± 0.008
Tx-11 (20%)[a]	3.458 ± 0.098	0.578 ± 0.008	0.290 ± 0.009	0.192 ± 0.007
Castor seed meal (10%)[b]	3.822 ± 0.167[c]	0.689 ± 0.029[c]	0.333 ± 0.010	0.231 ± 0.022

[a]Mean of six animals.
[b]Mean of five animals.
[c]Significantly more than control at $P < 0.05$.

TABLE 5

Mortality, Body Weight, Food Consumption and Food Conversion Ratio (±SE) at the End of Seven-Week Feeding Study

Group	Mortality	Initial weight (g)	Final weight (g)	Weight gain (g)	Food consumption (g)	Food efficiency ratio[a]
I. 0% Casein + 80% starch	4/4	33 ± 1	—	—	84	0
II. 2% Casein + 78% starch	3/4	33 ± 1	34	1	192	0.005
III. 4% Casein + 76% starch	0/4	33 ± 1	46 ± 6	13 ± 6	246 ± 24	0.053 ± 0.020
IV. 0% Casein + 80% Tx-11	0/4	33 ± 1	41 ± 4	8 ± 4	285 ± 4	0.028 ± 0.002
V. 2% Casein + 78% Tx-11	0/4	33 ± 1	70 ± 4[b]	37 ± 4[c]	380 ± 8[c]	0.097 ± 0.008[b]
VI. 4% Casein + 76% Tx-11	0/4	33 ± 1	93 ± 7[c]	60 ± 7[c]	450 ± 18[c]	0.133 ± 0.015[c]

[a]Weight increase per gram of food consumed.
[b]Significantly greater than corresponding values in Group III at $P < 0.02$.
[c]Significantly greater than corresponding values in Group III at $P < 0.01$.

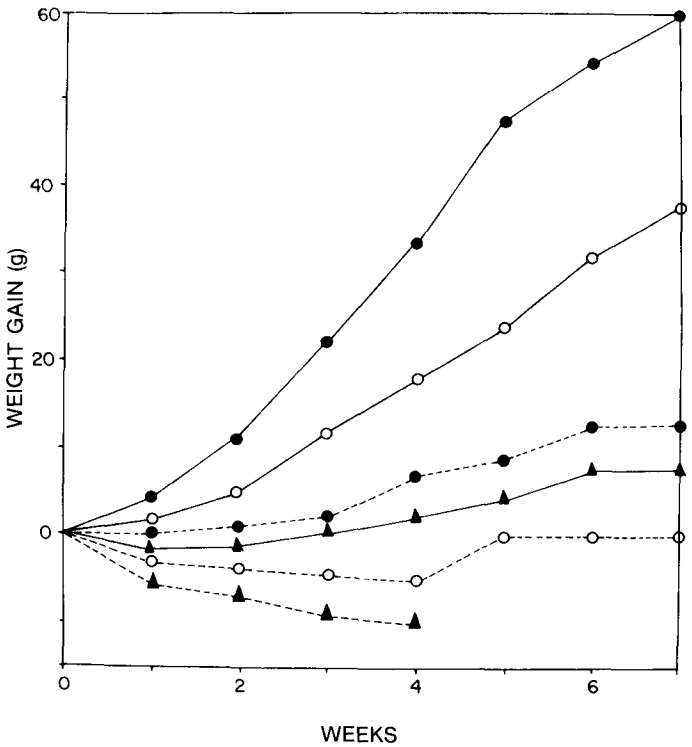

FIG. 3. Growth rate of rats fed with 76% Tx-11 + 4% casein (— ● —); 78% Tx-11 + 2% casein (—○—); 80% Tx-11 (—▲—); Control: (76% starch + 4% casein) (· · · ● · · ·), 78% starch + 2% casein (· · ·○· · ·) and 80% starch (· · ·▲· · ·).

group fed 80% Tx-11 (0% casein) were alive and showed a mean increase in body weight of 8 g. The only surviving animal from the control group fed the diet containing 78% starch + 2% casein showed a weight gain of only 1 g, whereas the corresponding experimental group fed 78% Tx-11 + 2% casein showed a mean increase in body weight of 37 g. In the groups fed diets containing 4% casein and 76% starch or 76% Tx-11, the mean weight increase in body weight was 13 and 60 g, respectively. The livers of rats fed the control diet containing 76% starch + 4% casein showed massive dissolution and atrophy of hepatocytes and enlarged vesicular nuclei. Their kidneys showed tubular and glomerular degeneration as well as disorganization of white pulp in the spleen. On the other hand, animals fed 76% Tx-11 + 4% casein presented an almost normal histology of the tissues. The animals of other groups fed Tx-11 (78% Tx-11 + 2% casein and 80% Tx-11 + 0% casein) showed varying degrees of damage in the tissues, but their intensity of damage was much less than that found in the animals fed 76% starch + 4% casein and 78% starch + 2% casein.

DISCUSSION

From the above studies, it was established that a 1:1 mixture of castor and sal seed meals could be fed to weanling rats without causing mortality. The *in vitro* studies carried out with aqueous extracts had indicated that a 1:0.6 ratio of castor seed meal and sal seed meal caused adequate neutralization by interaction of the toxins. However, to ensure complete detoxification of castor seed meal, excess sal seed meal was added because it is the lesser toxicant of the two. We felt that addition of ammonia for complexing with the unreacted tannins of the sal seed meal would render the castor + sal mixture more palatable. In an earlier study (14), we achieved the detoxification of sal seed meal by treatment with ammonia, and such ammoniated material appeared innocuous in feeding studies of rats. The toxic proteins, including ricin and allergens that were water-soluble, exhibited seven bands, and these were completely eliminated in the aqueous extract of the castor + sal seed meal mixture, as seen in the polyacrylamide gels. The aqueous extracts of castor seed meal produce a marked stimulating effect on smooth muscle such as guinea pig ileum and rat fundus. This effect was not observed with aqueous extracts of the castor + sal seed meal mixture. Recent studies by Fish and Thompson (15) have shown that red kidney bean lectin (which produces a potent inhibition of pancreatic amylase as well as hemagglutination of the red cells) when

preincubated with tannic acid not only reduced hemagglutinating activity but also had no effect on amylase-catalyzed digestion of starch.

The histopathological changes noted in the livers of rats fed raw castor seed meal were not observed in the livers of rats fed treated material. The animals fed Tx-11 at 76, 78 and 80% survived under the extremely stressful condition of deprivation of dietary protein (with low levels of casein 0–4%). Both untreated castor seed meal and sal seed meal, administered at half the levels of Tx-11 inclusion in the diet, would have caused 100% mortality in the rats (16,17). The experiment also reveals that an adequate amount of protein from castor seed meal was available, even after interaction with sal tannin, for sustenance of life. This was proved by the fact that all the four rats fed 80% starch (0% casein) died within one month, whereas those fed 80% Tx-11 (0% casein) not only survived the entire period of experimentation but had a healthy appearance and gained a mean body weight of 8 g during that period. The animals fed Tx-11 supplemented with 2 and 4% casein gained substantial weight as compared to the corresponding groups fed starch. Castor seed is deficient in the essential amino acids methionine, lysine and tryptophan (16). Supplementation of Tx-11 with protein containing these amino acids is essential for optimum growth.

Our experiments suggest that castor seed meal treated with sal seed meal is innocuous. Further feeding trials are necessary in farm animals to support its suitability as animal feedstuff.

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