Ricin: castor bean toxicosis

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ABSTRACT

The toxic principle of the castor bean plant, *Ricinus communis*, is a phytotoxin called ricin. The plant also contains the less toxic alkaloid ricinin, and an allergen or castor bean antigen C.B.A. Cases of castor bean toxicosis in sheep in Western Jamahirija have been recorded. The clinical and post mortem findings were described and the chemical and immunological similarities of ricin and ricinin were studied.

Results of recent electron microscopy studies, the effects of ricin on the living cell and the revelations of these studies on the mode of action of ricin are discussed.

INTRODUCTION

Castor bean is a tropical plant used ornamentally and as a source of castor oil. It is cultivated in loam to sandy loam soils in northern America as well as northern Africa, southern Europe, Argentina, Brazil, and other parts of the world (21).

Ricin is the toxic principle of *Ricinus communis*, the castor bean plant. It is a proteinaceous toxin or toxalbumin which is poisonous to humans and animals (3, 9, 10, 15) and is found in the seeds, leaves, and stem of the plant. The toxin is more concentrated in the seed. Since the time of ancient Egypt, the ingestion of castor bean seeds has been known to cause death. Seeds have been found in an Egyptian sarcophagos 4,000 years old. Herodotus and Pliny referred to castor oil in their writings. However, the toxicity of castor bean seeds was not explained until the eighteenth century (2).

Castor bean poisoning usually results from accidental ingestion of seeds or by feeding animals castor cake or «pomace», which still contains toxic residues following the extraction of castor oil. Pomace is used as feed or fertilizer because of its high nitrogen content (3, 21). Extracted castor oil does not contain ricin, and is used in the manufacture of pharmaceuticals and for other industrial purposes (3, 16, 17).

The plant is unpalatable. Livestock may eat it when other feed is lacking or when it is mixed with other foodstuffs. It is more palatable when less than 21 days old (18).

Review of Literature

Ricin has been found toxic to domestic animals (2, 3, 6, 9, 11, 15), humans (2, 9) and wild fowl (9). Clarke and Clarke (3) described the clincial signs in cattle which included hemorrhagic diarrhea and watery intestinal contents. Rath (16) also described trembling, weakness, pyrexia, incoordination, paralysis of hind limbs, muscular atrophy, hemorrhagic diarrhea with colic, nervous disturbances (from staggering, circular movements, to convulsion), heart failure and death in cattle. The lesions of ricin poisoning

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include patchy inflammation of the gastric and intestinal mucosa, hemorrhage and swelling of the mesentric lymph nodes. There was also oedema, frothy fluid accumulation in the trachea and bronchii together with swelling of the liver, spleen and kidney (3).

Chickens exhibit dullness, drooped wings, ruffled feathers and greyish-mottled wattles and combs (6). Jensen and Allen (9) found severe fatty changes in the liver and widely distributed patechial and ecchymotic hemorrhages of the intestinal tract of ducks.

The horse is apparently the most susceptible species to ricin poisoning. There is profuse sweating, tenesmus and tetanic-like spasms which involve especialy the hind limbs (3, 11, 15).

Fodstad, *et al.* (4) experimentally induced ricin intoxication by intravenous injection of purified ricin in mice and dogs. The mice shivered, became hypothermic and lost up to 20% of body weight. An accumulation of a clear fluid in the body cavities and congestion of the spleen were observed during necropsy. Dogs injected intravenously with low doses of ricin developed loss of appetite, slight elevation of of body temperature, and lost approximately 10% of body weight. Higher doses of ricin in dogs induced weakness, oedema of the extremities, ascites, melena, recumbency, and death within 15-40 hours. Lesions included congestion, petechial hemorrhages & hydrothorax.

There are several reports of castor bean poisoning in humans. Clarke (2) reported symptoms including vomiting, purging with intense pain, and tenderness of the epigastric region with cramps in the limbs. This progressed to low skin temperature and shock. The postmortem findings resembled those previously described in animals.

Chemical Characteristics of Ricin.

In 1958, Waller and Negi (20) extracted three substances from the castor bean plant, and related these substances to the adverse effects in animal and man:

1 - Ricin is a phytotoxin (toxalbumin) which is found primarily in seed husks. It is considered highly toxic, is a yellow-white water soluble powder. Remains in the pomace or castor cakes at a level of 3%, after oil extraction. The seed level is approximately 0.1%.
2 - Ricinin is an alkaloid which contains a cyanide radical.
3 - Allergen or castor bean antigen (CBA) is a protein — polysacchride complex.

All three substances are found in the plant seeds, leaves, and stem.

According to Salvedt (18) and Zimmerman *et al.* (21), Roberts and Stillmark first isolated ricin in 1880 and described it as a protein although it is degraded by neither trypsin nor pepsin. Because of its high molecular weight it is absorbed only slowly from the intestinal tract and can later be detected in milk, urine and other secretions (7, 16). An aqueous solution coagulates and turns to a harmless substance when boiled (18, 21).

Recently, the study of toxic principles in the castor bean plant has centered around ricin and ricinus agglutinin. Ricin comprises two polypeptide chains - an A effectomer (or isoleucine chain) and a B haptomer (or alanine chain). Ricinus agglutinin is considered to be less toxic and consists of two pairs of polypeptide chains (2 A′ chains and 2 B′ chains) which have some similarity to the ricin A and B chains (5, 6, 14, 18). Other names which have been given to ricinus agglutinin are ricin D and ricin variant. Earlier studies on the adverse effects caused by ricinus agglutinin were believed to be due to a ricin contaminant. However, subsequent experiments proved that ricinus agglutinin, although less toxic than ricin, also produce adverse effects. The A chain of ricin can inhibit about, 1,500 purified ribosomes per minute, while the A′ chain of ricinus agglutinin inactivates about 100 purified ribosomes per minute (14).

The A chain and A′ chain of ricin and ricinus agglutinin, respectively are both chemically and immunologically related, The B chain and B′ chain of these two toxins are not chemically related, but are immunologically similar. Saltvedt (18) concluded that the chains of both compounds were derived from the same ancestor molecule.

In the molecular pathogenesis of these toxins, the B chain binds the molecule to the cell surface. Both B and B′ chains have the ability to bind to terminal non-reducing galactose

Table 1 — Some properties of ricin and ricinus agglutinin*.

Property	Ricin	A-chain	B-chain	R. agg.	A′ chain	B′ chain
Mol. wt.	65,000	32,000	34,000	120,000	31,000	34,000
CHO content	4.5	2.6	6.4	4.6	—	—
Sensitivity to proteolytic enzyme	Low	high	high	low	—	—
LD_{50} in mice	65 ng	>10 ug	>10 ug	200 ug	—	—
Binding to cells	+	0	+	+	0	+
Inibition of cells-free protein	+	++	0	+	+	0
Mitogenic effect	toxic	0	+	toxic	—	—

*Modified from Olsnes, 1978.

residues associated with the cell wall. This binding can be inhibited by low concentrations of galactose or lactose. Both A and A′ chains inhibit the ribosomes of the cell nucleus (13, 14).

Mechanism of Action

Ricin was first thought to interfere with some energy-yielding process such as the tricarboxylic acid cycle in the hepatocytes. This interference would explain the decrease of plasma protein and glucose, liver and muscleglycogen and energy-rich phosphorus compounds in the liver. Consequently there is an accumulation of metabolic intermediate and end products such as lactic and pyruvic acids, inorganic phosphate, and nonprotein nitrogen (19). The necrotic changes in the alimentary tract mucosa alter permeability and are thought to facilitate the absorption of ricin (7).

Death from castor bean poisoning has been attributed to many causes including necrosis of parenchymal tissue, hemorrhage, and even cachexia. Heart failure may be involved as well as agglutination of erythrocytes leading to vascular thrombosis (6, 11, 16). Degenerative changes in the pyramidal cells of the cerebral cortex of the guinea pig have been described and similar changes may occur in the medulla leading to respiratory paralysis (7). Other researchers suggest that the lag time between ingestion and death indicates that the principal cause is protein synthesis inhibition and inactivation of various enzyme systems (4).

Combination of the above and perhaps other factors are probably involved.

Recently the cellular effects of ricin were summarized by Nicolson (12):

1 - Initial binding of the toxin at the cell surface.
2 - Subsequent entry into the cell by endocitosis.
3 - Transfer of the toxin into the cytoplasm.
4 - Inhibition of cell protein synthesis.

These events were studied with murine lymphoma BW 5147 $RIC^{R.3}$ sensitive cells and resistant variant cells. Although the resistant cells were 250 times more resistant to the cytotoxic effects and 1000 times more resistant to inhibition of protein synthesis, they still have a similar affinity for ricin. Ricin was not taken into the resistant cell but was internalized by the sensitive cell line. To study the dynamics of cellular transport, cell lines were labelled with Ferritin^{125}IRCAII at various concentrations. With this technique, affinity chromatography identified a galactoprotein receptor of 80,000 molecular weight in the plasma membrane of the sentitive cells but was absent in resistant cells. This was thought to be responsible for mediating the endocytotic incorporation of ricin. Another compound of a molecular weight of 70,000 was identified on the resistant cell membrane but was unassociated with ricin endocytosis.These studies led to the conclusion that the B-chain of the ricin binds to the 80,000 molecular weight membrane receptor site, and that cellular entry by ricin is via endocytosis, and toxic effects result from the inhibition of cellular protein synthesis by the ricin A-chain on the 60 S subunit (12).

Toxicity

The toxicity of ricin depends on many factors such as animal species, body weight and physiological condition, quantity ingested or route of administration. Comparative toxicological data regarding toxicity are listed in Table 2. (3, 4, 9).

Table 2 — Comparative susceptibility of farm animals to ricin poisoning.

Minimum lethal oral dose			
Horse	0.007 – 0.1 g/kg	Ox	2.0 g/kg
Man	0.18 g/kg	Calf	2.5 g/kg
Goose	0.4 g/kg	Duck	2.5 g/kg
Rabbit	1.0 g/kg	Goat	5.5 g/kg
Sheep	1.2 g/kg	Hen	14.0 g/kg
Pig	1.4 g/kg		

Minimum lethal intravenous dose	
Dog	1.5 - 1.75 ug/kg body weight
Mouse	2.7 ug/kg body weight

Diagnosis

The usual means of diagnosing castor bean poisoning are based on history, clinical signs and postmortem lesions. The labortory methods used to confirm the diagnosis are:

1 - microscopic examination of feed-stuffs and rumen or stomach contents for the presence of seeds and husks;
2 - erythrocyte agglutination test;
3 - precipitin tests (as with bacterial toxins);
4 - bioassay (feeding of of seeds and the experimental animals) (2, 3, 4, 8).

Ricin causes agglutination of rabbit and cattle RBC's in concentrations of 1:400,000 (16). Ricin incubated with rabbit leukocytes released endogenous pyrogens. This occurs in other mammals but not in cold blooded animals (1). Salmonellosis, arsenic poisoning, clostridial diseases, and other septicemias should be considered in the differential diagnosis.

Clinical observations.

Castor bean poisoning of sheep was seen by the senior author in the western region of Libya while practicing veterinary medicine. Most cases occurred on recently developed, irrigated farms in Jaffara were the castor bean plant was used as a border between farms. Sheep may eat the plant during the summer when grass forages are in short supply.

Owners first reported that animals were found rolling and hitting their heads on the ground and unable to stand. Sheep showed abdominal pain, they adopted a «tucked» posture and their fleece contained traces of sand from rolling. Most developed profuse salivation and diarrhea leading to dehydration, dryness of the mucous membranes, sunken eyes and shock. They appeared to be blind and the corneal reflex was lost. Other signs included tympany, increased respiration and heart rate, frequent urination

and watery diarrhea. Following treatment some animals recovered within 2-5 days. Treatment was on symptomatic lines and included giving of paraffin oil, atropin sulphate, daily intra-venous infusion of approximately 500 ml. of saline solution containing B vitamins mixed with electrolytes.

CONCLUSION

Ricin, the toxic principle of castor bean (*Ricinus communis*) is present throughout the plant but more concentrated in seeds. It is highly toxic to animals and man. After oil extraction, ricin remains in the castor cake or pomace which is sometimes used as a fertilizer or animal feed-stuff. The pomace normally contains about 3% ricin and about 8.8% of the less toxic compound ricinin. Pomace should be detoxified before being fed to animals. Castor bean plants and their by-products should be handled with care. Animals should not be allowed access to the castor bean plant since intoxication may occur from ingestion of any of its parts.

Ricin is bound to cell surface receptors and is taken up by endocytosis. It exerts its toxic affects by inhibiting protein synthesis in the cell through inactivation of the 60-S ribosomes sub-unit. Poisoned animals should be treated immediately and symptomatically with astringents and adsorbents to retard absorption from the alimentary tract. Antiricin serum should be available wherever poisong with castor bean is likely to occur.

ACKNOWLEDGEMENT

The authors thank Dr. T.M. Ali and Dr. D. Packer for their help in reviewing this paper and for their helpful suggestions.

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التسمم بنبـات الخـروع

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المستخلص

الرايسين هى المادة السامة الرئيسية فى نبات الخروع وهى عبارة عن تكسين نباتى. يحتوى نبات الخروع أيضاعلى مادتين أخريين أقل سمية وهما مادة الريسينين شبه القلوية ومولد الحساسية أو أنتيجين الخروع.

فى هذه الورقة سجلت حالات تسمم للأغنـام بمنطقـة غرب الجماهـيرية ووصفـت الأعراض الاكلينيكية ومشاهدات الصفة التشريحية للأغنام المصابة. كما وصف التشابه الكيماوى والمناعى بين مادتى الرايسين والرايسينين. ونوقشت أيضا نتائج دراسات المجهر الالكترونى الحديثة حول تأثير الرايسين على الخلية الحية وكذلك كشف النقاب عن طريقة عمل الرايسين فى الخلية.