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MECHANISMS OF RICIN POISONING: ISOLATION, MODE OF ACTION, AND IMPACT ON PROTEIN TRANSLATION

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ABSTRACT

Ricin, derived from Ricinus communis, is a potent toxin that inhibits protein synthesis and induces cell death via apoptosis and inflammatory responses. **Aim and Objective**: This review aims to elucidate ricin's mode of action, isolation techniques, and impact on translation, with objectives focused on understanding its structural basis of toxicity and its potential medical applications alongside biosecurity concerns. **Methodology**: A comprehensive analysis of literature covers ricin's isolation from castor seeds, protein purification steps, and studies on its biochemical action. The A chain's depurination of ribosomal RNA halts protein synthesis, while the B chain facilitates cell entry, both necessary for ricin's toxic effects. **Conclusion**: The dual-chain structure of ricin allows a precise mechanism of action, making it an effective cytotoxin with therapeutic promise in immunotoxins, yet a significant biosecurity risk due to its accessibility and ease of extraction. I**mplications**: The findings emphasize the need for safety protocols to address the risks of ricin misuse, while encouraging further research into ricin-based therapies. The study underscores the importance of balancing therapeutic potential with rigorous countermeasures to mitigate public safety risks.

INTRODUCTION

The castor oil plant, *Ricinus communis*, also known as Palma(e) Christi or wonder tree, is a perennial scrub belonging to the spurge family, Euphorbiaceae. It likely originated from Africa and has a rich history of use dating back to ancient civilizations such as Egypt, Rome and Greece. Nowadays, it grows wild in many tropical and subtropical regions and is cultivated as an ornamental plant worldwide.[1][2]

Throughout history, various parts of the castor oil plant, including the seeds and oil, have been utilized for diverse medical purposes. The oil, in particular, has been employed as a laxative and for the treatment of infections and inflammation. Its versatility and efficacy in traditional medicine have contributed to its continued use and cultivation across different cultures and regions.[1][3] The journey of understanding ricin's structure and function has been quite remarkable, starting from the early 1970s. At that time, researchers discovered that ricin consists of two chains, labelled A and B, which are linked by a disulfide bond.[4] This finding laid the foundation for further exploration into ricin's molecular architecture. Concurrently, scientists determined the complete primary sequence of both the A and B chains, providing essential insights into their composition.[5]

A significant breakthrough came when the structure of the ricin holotoxin was resolved at a resolution of 2.8 Å.[6] This pioneering work revealed that the ricin A chain is a globular protein with three domains, all contributing to its active site. In contrast, the B chain, acting as a lectin, folded into two domains, each capable of binding lactose within a shallow cleft. Notably, the interface between the A and B chains exhibited hydrophobic contacts, with proline and phenylalanine side chains playing crucial roles in this interaction.[7]

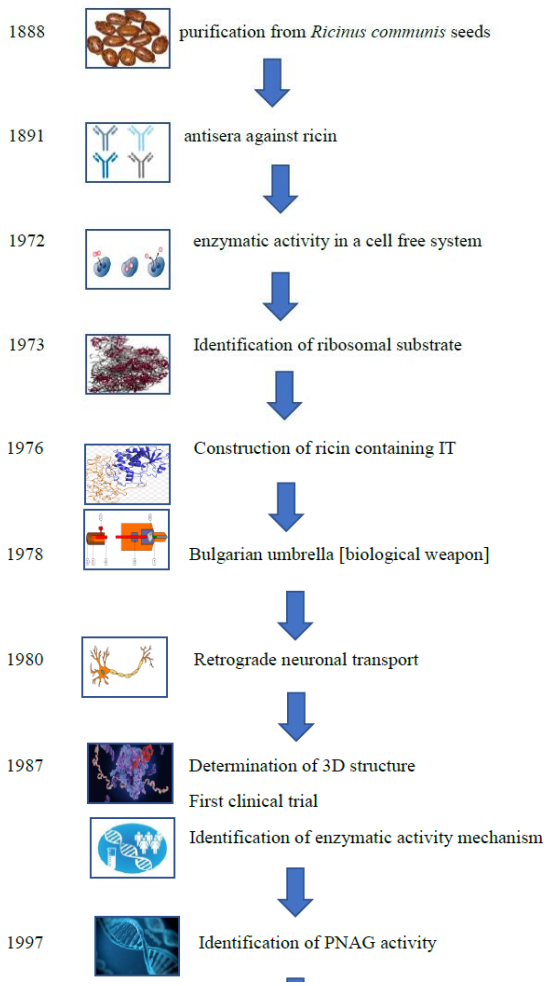
Building upon this foundational knowledge, researchers refined the structure of ricin four years later, achieving a resolution of 2.5 Å. This advancement allowed for a more detailed molecular description of both the holotoxin and its individual A and B chains. The ricin A chain, comprising 267 amino acids, adopts a globular structure organized into eight α-helices and eight β-strands. Similarly, the ricin B chain, consisting of 262 amino acids, features two homologous domains, each containing a lactose binding site. The presence of several areas of amino acid homology suggests a possible origin from gene duplication events.

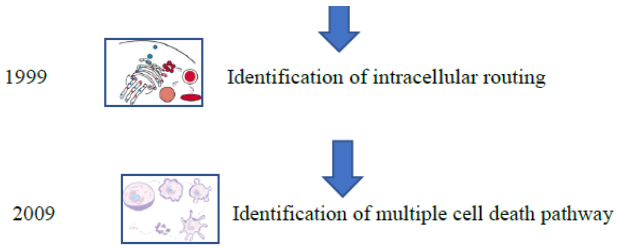
Further investigations in 1995 unveiled another facet of ricin's interaction, as researchers purified a complex of ricin A chain cross-linked to ribosomes. This discovery highlighted the binding of ricin A chain with ribosomal proteins L9 and L10e, shedding light on potential mechanisms of ricin toxicity.[8] Overall, the progressive unraveling of ricin's structure and interactions underscores the interdisciplinary efforts that have deepened our understanding of this potent toxin.[9]

Ricin, derived from castor beans, is extremely toxic and easily extractable, posing a significant threat for potential misuse in acts of bioterrorism or biological warfare. Its

accessibility from widely available sources necessitates strict regulation and ongoing efforts to develop detection methods and countermeasures to address the risk it presents.[10]

Promising treatments for ricin poisoning, including passive immunization with neutralizing antibodies and small-molecule compounds targeting ricin's intracellular trafficking, show potential in pre-clinical studies.[11][12] Recent data indicates that small-molecule compounds capable of interfering with or inhibiting ricin's intracellular trafficking may offer therapeutic benefits against ricinosis.[13] Combining antitoxin antibodies with immunomodulatory drugs has also demonstrated improved outcomes, but further research and clinical trials are needed for validation and development into clinically approved treatments.[14] Ricin toxic A chain (RTA) has been utilized therapeutically by conjugating it with ligands that target disease-causing cells, commonly employing cell-binding monoclonal antibodies (MAbs). These ligand-toxin conjugates, also known as immunotoxins (ITs), have demonstrated efficacy in clinical trials.[15][16] Our study aims to identify potential mode of action for ricin poisoning, isolation and its impact on the translation process.



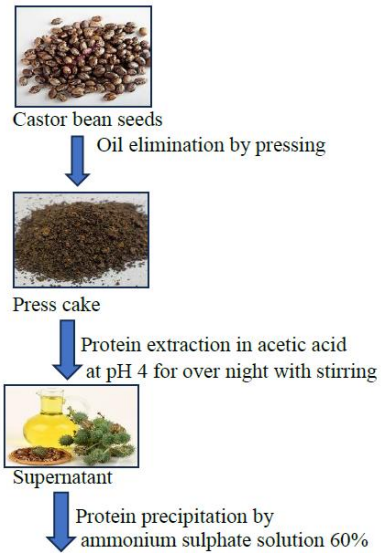


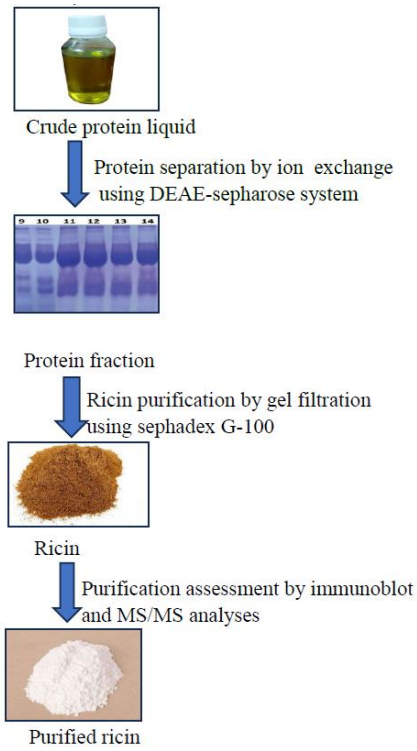
History

The toxicity of castor seeds was first studied at Schmiedeberg's laboratory in Strasbourg towards the end of the 1800s. It was possible to extract the toxic component of Ricinus using water and precipitate it with alcohol; however, heating, treatment with strong acid or repeated alcohol precipitation rendered the component non-toxic. Dixson postulated in 1887 that a toxic body resembling albumen was the cause of Ricinus's toxicity.[17] The Medical Faculty of Dorpat (now Tartu) found a partial purification of an exceedingly dangerous protein called ricin from castor seed or press cake, which solved the problem. This discovery was documented in Hermann Stillmark's PhD thesis, which he wrote under Prof. Rudolf Kobert's guidance. It was thought that ricin toxicity was caused by the agglutinating action of ricin on red blood cells until Stillmark observed that the agglutinin was distinct from the toxin.[18]

Isolation of ricin

The seeds of castor beans were properly cleansed, rinsed with water and then again with distilled water. Oil is extracted from washed seeds by pressing them firmly. After drying, the press cake was refluxed in 5% acetic acid with 1/10 (w/v) for 12 hours in order to extract proteins. After filtering the extract, a rotary flash evaporator was used to remove the solvent at 40±5 °C under decreased pressure.[19]





Protein Precipitation: Ammonium sulfate was added at various concentrations (30%, 40%, 50%, 60%, and 70%) to an acetic acid extract solution in order to precipitate total protein.

Protein Separation: The crude protein obtained from the precipitation step was subjected to ion exchange chromatography using DEAE-sepharose (Amersham Biosciences). The DEAE was equilibrated with 0.05 M Tris-HCl buffer at pH 8.0 to prepare the DEAE gel. A 20 mL crude protein solution was dissolved in 50 mL of DEAE gel and stirred for 15 minutes to allow protein adsorption. The protein-bound gel was then loaded onto a glass column (1.5 cm × 40 cm), washed with 100 mL of 0.05 M Tris-HCl buffer and allowed to stabilize for 30 minutes. Desorption was carried out using 0.05 M Tris-HCl buffer at pH 8.0, supplemented with 0.05 M NaCl, at a flow rate of 0.2 mL/min. Protein fractions were collected in 2 mL aliquots.

Protein Purification: Ricin was purified via gel filtration using Sephadex G-100 (Amersham Biosciences). The gel was first activated in distilled water (1 g in 10 mL H_2O) for 6 hours at room temperature, loaded into a glass column (2.5 cm × 50 cm) and equilibrated with 0.002 M phosphate buffer at pH 7.2, with a protein solution-to-gel ratio of 1:6 (v/v). Protein fractions were eluted at a flow rate of 0.2 mL/min and collected in 2 mL volumes.[20,21]

Mode of toxic action

Type-II ribosome-inactivating proteins (RIPs) represent a significant class of protein toxins, typically comprised of A and B chains linked by an inter-chain disulfide bond. It has long been established that isolated A and B chains of RIPs are not toxic on their own.[22] Recent research has elucidated that the B-chain, with its lectin-like activity, plays a crucial role in binding to galactose-containing receptors present on the surfaces of eukaryotic cells. This binding is essential for facilitating the internalization of the A-chain via endocytosis. Following endocytic uptake, translocation of the A-chain occurs through retrograde traffic, involving passage through the trans-Golgi network and the endoplasmic reticulum.[23] Indeed, the A-chain of type-II ribosome-inactivating proteins (RIPs) possesses N-glycosidase activity, which catalyzes the irreversible depurination of adenine from the 28S ribosomal RNA. This enzymatic activity results in the termination of protein synthesis within the affected cells. When combined with the lectin-like activity of the B-chain, this synergism enables the internalization of the A-chain into cells, leading to high cytotoxicity following oral administration or inhalation.[24]

Mechanism of induced- apoptosis

Although ricin-mediated rRNA depurination event effectively inhibits protein synthesis, it cannot be concluded that these mechanisms cause cell death on their own.[25,26,27] It has been shown that ricin can cause the production of inflammatory mediators, autophagy and apoptosis.[28,29] These mechanisms have been investigated for more than 20 years and an increasing amount of data suggests that they are important for the observed cell death. Nevertheless, it is still unknown to what degree additional variables are necessary for the activation of apoptosis and whether the suppression of protein synthesis is enough to cause it in all cell types.[30]

It is believed that caspases, members of the Bcl-2 family[31,32] and stress-associated signaling pathways are the primary mechanisms underlying ricin-dependent apoptosis.[33,34,35,36] The processes of ricin-induced cell death-promoting pathways are also linked to ricin's direct and indirect effects on DNA[37] its ability to produce reactive oxygen species, and its ability to trigger apoptosis in ricin B-chain.[38]

Ricin A-chain induced apoptosis

An N-glycosidase called ricin A-chain eliminates a conserved adenine from the α-sarcin-ricin loop (SRL) of the rRNA found in the large ribosomal subunit at position 4324 in human cells

and A3027 in yeast.[39,40,41,42] This prevents certain elongation factors from attaching to the ribosome and prevents the production of new proteins.[43] The structure of ribosomal RNA is known to be influenced by the ricin A-chain. It modifies the GTPase activating centre (SRL) of the ribosome's dynamic flexibility. The elongation cycle's transition between the pre and post-translocational phases is disrupted by these conformational alterations.[44] suggesting that for the proper catalytic activity of ricin, the whole ribosome and particularly ribosomal proteins are crucial.[45] It has been shown that in eukaryotes, the ribosomal stalk shape promotes ricin A-chain interaction with the big ribosomal subunit.[46,47] The three different phosphoprotein types that make up the human ribosomal stalk structure are P0, P1 and P2. They come together to form a pentameric protein complex, which is made up of two heterodimers of the P1 and P2 proteins coupled to a single P0 protein[48,49] Recent research has demonstrated that P1–P2 proteins serve as the main binding site for RTA, with the P1B–P2A dimer playing a more crucial role.[50,51]

Ricin B-chain induced apoptosis

Research conducted on U937 cells has shown that the interaction between membrane glycoproteins and glycolipids and ricin B-chain may initiate signaling processes that result in apoptosis. This lectin activity-dependent process was different from the ricin A-chain-induced apoptosis signaling pathways. It has been shown that the characteristic apoptotic nuclear morphological alterations and DNA fragmentation that were seen in ricin-treated cells were caused by the carboxymethylated (CM-) ricin B-chain.[52] In U937 cells, CM-ricin B-chain was unable to prevent protein production. Therefore, at least in U937 cells, our studies provide credence to the idea that ricin-induced apoptosis or at least some of the apoptotic pathways are independent and unrelated to the inhibition of protein synthesis. Results that were recently published additionally demonstrate that the A-chain is not the only factor responsible for ricin-induced apoptosis.[53] Rats were given intact heterodimeric ricin and ricin chains to investigate ricin-induced apoptosis in the liver, which is a significant location of ricin uptake and cytotoxicity in vivo.[54] It was shown that RTB induced the expression of TNF-α, IL-6 and inducible nitric oxide synthase (iNOS), all of which are implicated in the activation of JAK-STAT, NF-κB and protein tyrosine kinase.[55]

Toxic Action of Ricin on The Translational Process

Ricin holotoxin, comprising A and B subunits, lacks catalytic activity towards ribosomes; while the A chain possesses ribosome-inactivating N-glycosidase activity, it's typically

suppressed in the holotoxin form, with the B chain facilitating cell binding and internalization.[56] When ricin A chain (RTA) is dissociated from ricin B chain (RTB), a cluster of arginine residues positioned at the interface domain between RTA and RTB becomes exposed to the solvent, providing an interaction platform for P-stalk proteins. This interaction between RTA and the P-stalk proteins stimulates the toxin, enabling it to activate its enzymatic activity by orienting the active site of RTA, opposite to the arginine interface, toward the sarcin-ricin loop (SRL) of the ribosome.[57] RTA functions as an RNA N-glycosidase, catalyzing the hydrolysis of the N-glycosidic bond between a specific adenine residue on the sarcin-ricin loop (SRL) of ribosomal RNA and the sugar backbone.[58] RTA catalyzes the hydrolysis of the N-glycosidic bond between a specific adenine residue on the sarcin-ricin loop (SRL) of ribosomal RNA and the sugar backbone. This specificity in rRNA depurination is influenced by the conformation of the SRL loop structure, with the GAGA sequence, featuring a prominent adenine base, recognized as a major element in this process.[59] During the catalysis of the sarcin-ricin loop (SRL) depurination process by ricin, the conserved adenine located at the tip of the SRL is inserted between two tyrosine residues (Tyr80 and Tyr123) within the catalytic center of ricin A chain (RTA). This positioning facilitates π-stacking interactions between the adenine base and the aromatic rings of the tyrosine residues, contributing to the catalytic activity of RTA in hydrolyzing the N-glycosidic bond of the adenine residue.[60] In addition to the π-stacking interactions with tyrosine residues, the adenine position during SRL depurination is stabilized by hydrogen bonding with specific residues within RTA. These include Gly121, Val81, Glu177 and Arg180, which contribute to the overall stability of the enzyme-substrate complex and facilitate the catalytic activity of RTA in hydrolyzing the N-glycosidic bond of the adenine residue.[61] Studies have demonstrated that two specific residues within RTA, Glu177 and Arg180, play a critical role in the hydrolysis of N-glycosidic bonds by stabilizing the transition state during the catalysis of the depurination reaction. These residues contribute to the catalytic mechanism by facilitating interactions that stabilize the transition state, thereby promoting the efficient cleavage of the N-glycosidic bond between the adenine base and the sugar backbone of the ribosomal RNA.[62]

In the 1970s, it was observed that ricin inhibits translation in mammalian cells[63], a finding that was later confirmed using in vitro experimental systems. This discovery highlighted ricin's ability to disrupt protein synthesis, a key mechanism underlying its cytotoxic effects.[64][65]

CONCLUSION

Ricin, derived from the castor oil plant *Ricinus communis*, remains one of the most potent naturally occurring toxins known. Its dual-chain structure, with the A chain acting as a ribosome-inactivating protein and the B chain facilitating cell entry, underscores its highly specialized toxic action. The elucidation of its molecular structure, particularly the A chain's role in catalyzing the depurination of ribosomal RNA, has advanced our understanding of its mechanism of action, which ultimately halts protein synthesis. Ricin-induced cell death involves complex processes, including apoptosis and oxidative stress, making its toxicological impact multifaceted. Furthermore, the historical development of therapeutic interventions, including immunotoxins and small-molecule inhibitors, highlights the potential for ricin's application in medical settings, although its misuse in bioterrorism remains a significant concern. Ongoing research into the intricate pathways of ricin-induced toxicity, its interactions with ribosomal and other cellular components, and the development of effective countermeasures will be critical in addressing the dual challenges of therapeutic potential and public safety.

REFERENCE

1. Scarpa A, Guerci A. Various uses of the castor oil plant (Ricinus communis L.). A review. J Ethnopharmacol, Mar. 1982; 5(2): 117-37. PMID: 7035750.
2. Nicholson, Paul T., and Ian Shaw, eds. Ancient Egyptian materials and technology. Cambridge University Press, 2000.
3. Worbs S, et al. Ricinus communis intoxications in human and veterinary medicine—a summary of real cases. Toxins (Basel), Oct. 2011; 3(10): 1332-72. PMID: 22069699; PMCID: PMC3210461.
4. Olsnes S, Pihl A. Different biological properties of the two constituent peptide chains of ricin, a toxic protein inhibiting protein synthesis. Biochemistry, Jul. 31, 1973; 12(16): 3121-6. PMID: 4730499.
5. Funatsu G, Yoshitake S, Funatsu M. Primary structure of lie chain of ricin D. Agricultural and Biological Chemistry, 1978; 42(2): 501-503.
6. Montfort W, et al. The three-dimensional structure of ricin at 2.8 A. J Biol Chem., Apr. 15, 1987; 262(11): 5398-403. PMID: 3558397.
7. Rutenber E, et al. Crystallographic refinement of ricin to 2.5 Å. Proteins: Structure, Function, and Bioinformatics, 1991; 10(3): 240-250.

8. Vater CA, et al. Ricin A chain can be chemically cross-linked to the mammalian ribosomal proteins L9 and L10e. J Biol Chem., May 26, 1995; 270(21): 12933-40. PMID: 7759553.
9. Polito L, et al. Ricin: An Ancient Story for a Timeless Plant Toxin. Toxins (Basel), Jun. 6, 2019; 11(6): 324. PMID: 31174319; PMCID: PMC6628454.
10. Guo Z, et al. Proteomic study of differential protein expression in mouse lung tissues after aerosolized ricin poisoning. Int J Mol Sci., Apr. 28, 2014; 15(5): 7281-92. PMID: 24786090; PMCID: PMC4057672.
11. Noy-Porat T, et al. Extended therapeutic window for post-exposure treatment of ricin intoxication conferred by the use of high-affinity antibodies. Toxicon, Mar. 1, 2017; 127: 100-105. PMID: 28089771.
12. Noy-Porat T, et al. Isolation of Anti-Ricin Protective Antibodies Exhibiting High Affinity from Immunized Non-Human Primates. Toxins (Basel), Mar. 3, 2016; 8(3): 64. PMID: 26950154; PMCID: PMC4810209.
13. Gupta N, et al. Inhibitors of retrograde trafficking active against ricin and Shiga toxins also protect cells from several viruses, Leishmania and Chlamydiales. Chem Biol Interact, Apr. 1, 2017; 267: 96-103. PMID: 27712998.
14. Gal Y, et al. Potent antiedematous and protective effects of ciprofloxacin in pulmonary ricinosis. Antimicrobial Agents and Chemotherapy, 2016; 60(12): 7153-7158.
15. Pincus SH, et al. Passive and active vaccination strategies to prevent ricin poisoning. Toxins (Basel), Sep. 2011; 3(9): 1163-84. PMID: 22069761; PMCID: PMC3202875.
16. Pastan I, et al. Immunotoxin treatment of cancer. Annu Rev Med., 2007; 58: 221-37. PMID: 17059365.
17. Franz H. The ricin story. Adv Lectin Res., 1988; 1: 10-25.
18. Takahashi T, et al. Biochemical Studies on Castor Bean Hemagglutinin: I. Separation and Purification. The Journal of Biochemistry, Apr. 1, 1962; 51(4): 288-92.
19. Trung NN, et al. Effects of ricin extracted from seeds of the castor bean (Ricinus communis) on cytotoxicity and tumorigenesis of melanoma cells. Biomedical Research and Therapy, Jun, 2016; 3: 1-2.
20. Jang HY, Kim JH. Isolation and biochemical properties of ricin from Ricinus communis. Korean Biochemical Journal, 1993; 26(1).
21. Kumar O, et al. Purification and biochemical characterisation of Ricin from castor seeds. Defence Science Journal, May 1, 2004; 54(3): 345.

22. Creppy EE, et al. Action on ricin and its polypeptide chains on cultured hepatoma cells. Arch Toxicol, Mar. 1980; 44(1-3): 175-9. PMID: 7387399.
23. Sowa-Rogozińska N, et al. Intracellular Transport and Cytotoxicity of the Protein Toxin Ricin. Toxins (Basel), Jun. 18, 2019; 11(6): 350. PMID: 31216687; PMCID: PMC6628406.
24. Liang L, et al. Highly toxic type II ribosome-inactivating proteins ricin and abrin and their detection methods: a review. Se Pu., Mar. 2021; 39(3): 260-270.
25. Słomińska-Wojewódzka M, Sandvig K. Ricin and ricin-containing immunotoxins: insights into intracellular transport and mechanism of action in vitro. Antibodies, Apr. 19, 2013; 2(2): 236-69.
26. Baluna R, et al. The effect of a monoclonal antibody coupled to ricin A chain-derived peptides on endothelial cells in vitro: insights into toxin-mediated vascular damage. Experimental Cell Research, Aug. 1, 2000; 258(2): 417-24.
27. de Souza LM, et al. Cell toxicity by ricin and elucidation of mechanism of Ricin inactivation. International Journal of Biological Macromolecules, Jul. 1, 2018; 113: 821-8.
28. Day PJ, et al. Binding of ricin A-chain to negatively charged phospholipid vesicles leads to protein structural changes and destabilizes the lipid bilayer. Biochemistry, Feb. 26, 2002; 41(8): 2836-43.
29. Kumar O, et al. Oxidative stress associated hepatic and renal toxicity induced by ricin in mice. Toxicon, Mar. 1, 2003; 41(3): 333-8.
30. Bagaria S, Karande A. Abrin and immunoneutralization: A review. Toxinology; Gopalakrishnakone, P., Ed.; Springer: Dordrecht, The Netherlands, 2014; 1-21.
31. Brinkmann U, et al. Effects of BCL-2 overexpression on the sensitivity of MCF-7 breast cancer cells to ricin, diphtheria and Pseudomonas toxin and immunotoxins. Apoptosis, Jan. 1997; 2: 192-8.
32. Tamura T, et al. Resistance against ricin-induced apoptosis in a brefeldin A-resistant mutant cell line (BER-40) of Vero cells. The Journal of Biochemistry, Sep. 1, 2002; 132(3): 441-9.
33. Horrix C, et al. Plant ribosome-inactivating proteins type II induce the unfolded protein response in human cancer cells. Cellular and Molecular Life Sciences, Apr. 2011; 68: 1269-81.
34. Wang CT, et al. Inhibition of the unfolded protein response by ricin a-chain enhances its cytotoxicity in mammalian cells. Toxins, May 10, 2011; 3(5): 453-68.

35. Higuchi S, et al. Cross-talk between the pathways leading to the induction of apoptosis and the secretion of tumor necrosis factor-α in ricin-treated RAW 264.7 cells. The Journal of Biochemistry, Dec. 1, 2003; 134(6): 927-33.
36. Jandhyala DM, et al. ZAK: a MAP3Kinase that transduces Shiga toxin and ricin-induced proinflammatory cytokine expression. Cellular Microbiology, Jul. 2008; 10(7): 1468-77.
37. Barbieri L, et al. Ribosome-inactivating proteins depurinate poly (ADP-ribosyl) ated poly (ADP-ribose) polymerase and have transforming activity for 3T3 fibroblasts. FEBS Letters, Mar. 13, 2003; 538(1-3): 178-82.
38. Authier F, et al. Fate and action of ricin in rat liver in vivo: translocation of endocytosed ricin into cytosol and induction of intrinsic apoptosis by ricin B-chain. Cellular Microbiology, Dec. 2016; 18(12): 1800-14.
39. Hasegawa N, et al. Isolated ricin B-chain-mediated apoptosis in U937 cells. Bioscience, Biotechnology, and Biochemistry, Aug. 2011; 75(8): 1578-83.
40. Wang Y, et al. Ricin-induced apoptosis in RAW 264.7 cells involves reactive oxygen species generation and mitochondrial dysfunction. Toxicol Appl Pharmacol, Nov. 1, 2009; 239(2): 141-50.
41. Wang Y, et al. A study on the mechanism of ricin-induced apoptosis in RAW264.7 cells. Toxicol Appl Pharmacol, Jan. 1, 2010; 243(1): 59-66.
42. Wang Y, et al. Ricin-induced cytotoxicity in the human U937 cell line: Role of the intracellular calcium signaling. Toxicol Lett., Jan. 20, 2012; 208(2): 189-94.
43. Orta-Zavalza E, et al. Identification of ricin toxins in the red blood cells of a patient with suspected ricin poisoning. J Forensic Sci., May 2016; 61, 1. PMID: 27043192.
44. Fink A, et al. Cloning and characterization of the ricin A-chain from Ricinus communis. Protein Science, Dec. 1998; 7(12): 2491-8.
45. Guo Z, et al. The role of mitochondrial dysfunction in the cytotoxicity of ricin. Toxins (Basel), Aug. 2017; 9(8): 221.
46. Wang Y, et al. Mechanism of ricin-induced cytotoxicity in human lymphocytes: the role of oxidative stress. Toxicol Lett., Jan. 15, 2011; 202(2): 88-94.
47. Wu Y, et al. Rutin protects against ricin-induced apoptosis in human embryonic kidney (HEK293) cells through inhibition of oxidative stress. J Nutr Biochem, Jan. 2014; 25(1): 81-90.
48. Larsson SL, Sloma MS, Nygård O. Conformational changes in the structure of domains II and V of 28S rRNA in ribosomes treated with the translational inhibitors ricin or α-sarcin.

Biochimica et Biophysica Acta (BBA)-Gene Structure and Expression, Aug. 19, 2002; 1577(1): 53-62.

49. May KL, Yan Q, Tumer NE. Targeting ricin to the ribosome. Toxicon, Jul. 1, 2013; 69: 143-51.
50. Chiou JC, Li XP, Remacha M, Ballesta JP, Tumer NE. The ribosomal stalk is required for ribosome binding, depurination of the rRNA and cytotoxicity of ricin A chain in Saccharomyces cerevisiae. Molecular Microbiology, Dec. 2008; 70(6): 1441-52.
51. May KL, Li XP, Martínez-Azorín F, Ballesta JP, Grela P, Tchórzewski M, Tumer NE. The P1/P2 proteins of the human ribosomal stalk are required for ribosome binding and depurination by ricin in human cells. The FEBS Journal, Oct. 2012; 279(20): 3925-36.
52. Grela P, Sawa-Makarska J, Gordiyenko Y, Robinson CV, Grankowski N, Tchórzewski M. Structural properties of the human acidic ribosomal P proteins forming the P1–P2 heterocomplex. Journal of Biochemistry, Feb. 1, 2008; 143(2): 169-77.
53. Lee KM, Yu CW, Chiu TY, Sze KH, Shaw PC, Wong KB. Solution structure of the dimerization domain of the eukaryotic stalk P1/P2 complex reveals the structural organization of eukaryotic stalk complex. Nucleic Acids Research, Apr. 1, 2012; 40(7): 3172-82.
54. Grela P, Li XP, Horbowicz P, Dźwierzyńska M, Tchórzewski M, Tumer NE. Human ribosomal P1-P2 heterodimer represents an optimal docking site for ricin A chain with a prominent role for P1 C-terminus. Scientific Reports, Jul. 17, 2017; 7(1): 5608.
55. Grela P, Li XP, Tchórzewski M, Tumer NE. Functional divergence between the two P1–P2 stalk dimers on the ribosome in their interaction with ricin A chain. Biochemical Journal, May 15, 2014; 460(1): 59-69.
56. Spooner RA, Watson PD, Marsden CJ, Smith DC, Moore KA, Cook JP, Lord JM, Roberts LM. Protein disulphide-isomerase reduces ricin to its A and B chains in the endoplasmic reticulum. Biochem J., Oct. 15, 2004; 383(Pt 2): 285-93.
57. Li XP, Kahn PC, Kahn JN, Grela P, Tumer NE. Arginine residues on the opposite side of the active site stimulate the catalysis of ribosome depurination by ricin A chain by interacting with the P-protein stalk. J Biol Chem., Oct. 18, 2013; 288(42): 30270-30284.
58. Endo Y, Tsurugi K. The RNA N-glycosidase activity of ricin A-chain. The characteristics of the enzymatic activity of ricin A-chain with ribosomes and with rRNA. J Biol Chem., Jun 25, 1988; 263(18): 8735-9.

59. Glück A, Endo Y, Wool IG. Ribosomal RNA identity elements for ricin A-chain recognition and catalysis. Analysis with tetraloop mutants. J Mol Biol., Jul. 20, 1992; 226(2): 411-24.
60. Jasheway K, Pruet J, Anslyn EV, Robertus JD. Structure-based design of ricin inhibitors. Toxins (Basel), Oct. 2011; 3(10): 1233-48.
61. Wahome PG, Robertus JD, Mantis NJ. Small-molecule inhibitors of ricin and Shiga toxins. Curr Top Microbiol Immunol, 2012; 357: 179-207.
62. Kim Y, Robertus JD. Analysis of several key active site residues of ricin A chain by mutagenesis and X-ray crystallography. Protein Eng., Dec. 1992; 5(8): 775-9.
63. Lin JY, Kao WY, Tserng KY, Chen CC, Tung TC. Effect of crystalline abrin on the biosynthesis of protein, RNA, and DNA in experimental tumors. Cancer Res., Sep. 1970; 30(9): 2431-3.
64. Osborn RW, Hartley MR. Dual effects of the ricin A chain on protein synthesis in rabbit reticulocyte lysate. Inhibition of initiation and translocation. Eur J Biochem, Oct. 24, 1990; 193(2): 401-7.
65. Grela P, Szajwaj M, Horbowicz-Drożdżal P, Tchórzewski M. How Ricin Damages the Ribosome. Toxins (Basel), Apr. 27, 2019; 11(5): 241.