HIGH SCHOOL EDITION
Journal of Student Research
Volume 11 Issue 3 (2022)

A Review of Extraction and Detection of Ricin from Castor Plant and the Effect of Ricin on Humans

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ABSTRACT

Ricin is a ribosome inactivating protein produced within the seeds of the castor oil plant (Ricinus communis). Ricin can be extracted from the mash of crushed castor seeds using different procedures. Ricin has 2 chains, the A chain and B chain. It is extremely potent to mammals, being able to kill 1500 cells per minute with small doses. Castor bean plants grown as an ornamental plant and are found in most households in parks around India, eastern Africa, and the south-eastern Mediterranean Basin. Methods of detection for ricin have been found, but not one that takes into account of its presence in biological fluids. Ricin has also been found to be an immuno-toxin against cancer, and it could be used as a medication in the future.

Introduction

Ricin (Nick Verougstraete, Dries Helsloot, 2019) is a carbohydrate-binding protein produced in the seeds of the castor oil plant, also known as Ricinus communis. Ricin is a highly potent toxin that's lethal in small doses. The median lethal dose of ricin is around 22 micrograms per kilogram of the body (tested on mice's) weight via intraperitoneal injection. Oral exposure to ricin is less toxic. The estimated lethal oral dose in humans is approximately 1 milligram per kilogram of body weight. Inhalation is the most potent, with just 5-10 micrograms per kg for adults and even less for adolescents. The survival rate for critical damage is 2-6% and for mediocre damage is 5-15%.

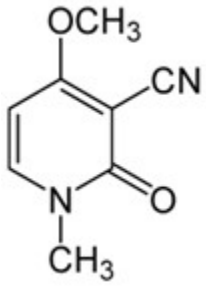

Figure 1. Ricin particle

Ricinus communis is indigenous to the south-eastern Mediterranean Basin, India, and eastern Africa (Ngo, T., Nguyen, T., 2016). Today it is widespread throughout all tropical regions as an ornamental plant. Ricinus communis establishes itself in suitable climates where it can become an invasive plant and is often found on wastelands.

It is used extensively as a decorative plant in parks and houses. Suppose sown early and kept under glass while maintaining at a temperature of around 20 C (68 F) until germinated. The castor oil plant can reach a height of 2–3 meters (6.6–9.8 ft) within a year. In areas of frost, it is prone to grow less. However, it can grow

well outdoors in cooler climates, at least in southern England, and the leaves do not appear to be affected by frost damage in covered spots, where it remains green.

Castor beans are used to produce castor oil and ricin. Castor oil is used as a highly effective laxative, but it also contains antiviral and antimicrobial properties (mainly for dermatosis and fungal infections). Castor oil is extracted from the mash and leftover after grinding castor beans. Organic solvents such as hexanes, acids, or acetone are used to extract the oil. The mash also contains ricin, which is leftover from the extraction. A salting-out procedure is used to extract the toxin from the mash.

An incident related to ricin is based on Georgi Markov. Georgi Markov was a Bulgarian journalist who had former knowledge of the previous communist leader. While in London, KGB officer Todor Zhivkov poked Georgi's leg with the tip of an umbrella. 4 days later, Georgi succumbed to his injuries at the age of 49. The tip of the umbrella was laced with 500 micrograms of ricin. Georgi had symptoms of fever, nausea, tachycardia, and vomiting. On his second day, he was recorded to have 160 BPM, and he was hypotensive and leukocytic. Doan, Leah G. (2004)

Toxicity of ricin

Ricin is a potent toxic which inactivates ribosomes for mammals. The toxicity varies from species to species; horses specifically are susceptible, and frogs and chickens are less so. The A chain (RTA) attaches to 28S ribosomal RNA in the 60S ribosomal subunit and halts protein elongation. One ricin molecule can kill an entire cell, and a small portion of ricin's A chain can inactivate 1500-2000 ribosomes per minute. The b chain (RT) molecules are transported to endosomes of the trans-Golgi network and then the endoplasmic reticulum.

RT is absorbed within 2 hours via blood and lymphatic vessels when ingested, and it accumulates within the spleen and liver. This causes GI haemorrhage and liver, spleen, and kidney necrosis. There is severe localized pain and necrosis of the regional lymph nodes and muscles when injected intramuscularly. It also causes calcium and magnesium imbalances, the release of cytokines, and oxidative stress in the liver, and with an adequate dose, it will cause hepatic failure. Even up to 1:10,000 dilutions of RT are adequate enough to induce pseudomembranous conjunctivitis and conjunctival irritation. Symptoms may occur five days after exposure, even in previously asymptomatic patients.

Ricin in the system

Ricin poisoning has no specific symptoms. There are varying ranges of intensity when it comes to specific symptoms similar to other toxins and pulmonary diseases. The intensity is dependent on the subject, dose, and area of delivery. There is no guaranteed or certified method to deduce ricin poisoning, but examining symptoms, probable cause, and situational variables.

Ricin has been detected in animal tissues through enzyme-linked immunosorbents, suggesting that injected ricin is detectable (but a well-developed method for inhalation does not exist). Ricin can be detected in rat urine 48 h after ricin exposure, suggesting that ingestion of laced drinks and foods can be detected soon in humans.

The damage caused by ricin is irreversible as it causes direct cell death. Only supportive treatments such as intravenous fluids, oxygen masks, and medications that lower blood pressure (to lower circulation) can reduce the effect of ricin and increase the survival rate of the subject. (Mohammad Moshiri, L., 2016)

New research on Dexamethasone and difluoromethylornithine suggests that survival rates of intoxicated mice can be increased with exposure to the following chemicals (Doan, Leah G., 2004). There are antibodies against rt and RTB, and they prevent binding, internalization, or routing of ricin to the endosomal compartment. The antibodies are said to protect animals for 8-12 hours.

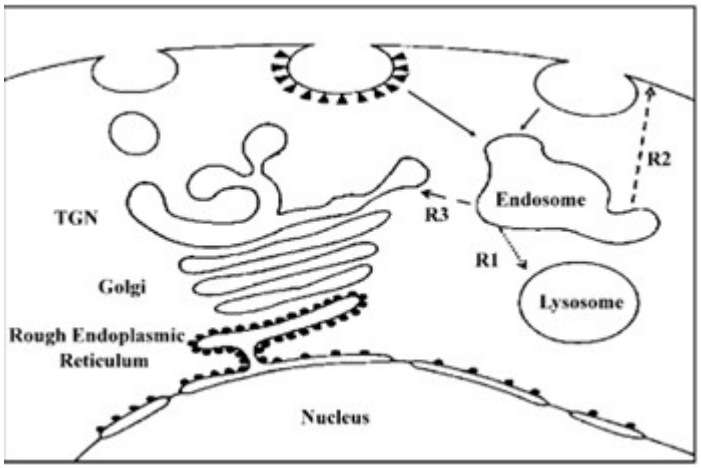

Figure 2: Ricin's entry and course through a cell

Method of extraction

Method:1

The first method of extraction (Ngo, T., Nguyen, T., 2016) cleans the seeds and washes them twice, once in the water, then rewashed in distilled water. The seeds were kept under an unspecified higher amount of pressure to remove any oil, and then the leftover mash was dried and refluxed in acetic acid 5% with 1/10 (w/v) for 12 hours to extract the proteins of ricin. The extract was filtered, then the solvent was evaporated under lower pressures and 40±5 C using a rotary flash evaporator. This method does not specify the pressure, and the varying temperatures of ±5 C could result in different outcomes and different yields.

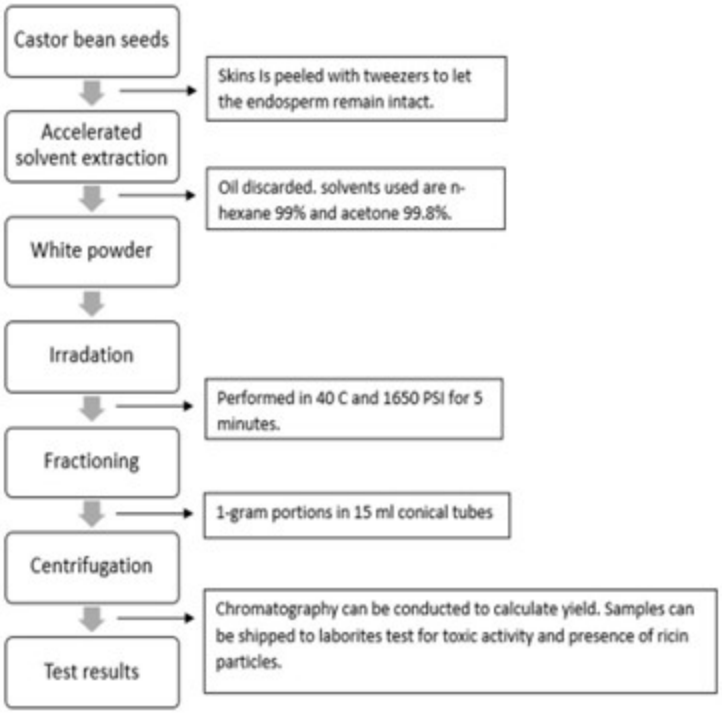

Figure 3: The steps to extract ricin from Castor beans. This is specified in the second method listed below**.**

Method:2

The method followed by (Sousa, Roberto B.; Lima, Keila S. C., 2019) uses a spatula or tweezers to peel castor beans until the endosperm is exposed. Mill remaining beans until homogenized into a mash. Transfer mash into the heating element of the extractor. Perform extraction with a mixture of 8:2 solvents n-hexane 99% UV/HPLC and acetone 99.8% HPLC. Pump mixture into the cell and raise pressure to 1650 psi at the constant temperate of 40 C for 5 mins. Gather extract and filter it over a flask for collection. Purge cell with nitrogen for 1 min and repeat procedure for more samples. The white powder is ricin. If further testing is required to ascertain the presence of toxic activity or intact ricin particles, gather all samples, and homogenize them. Separate them into fractions of 1 g. Pack each fraction into 15 ml conic tube and centrifugate them. Place each tube into plastic bags.

The contents in the liquid can be used to calculate the yield through the process of chromatography. For further details such as toxic activity and the presence of ricin particles, the samples can be shipped to laboratories to have tests conducted on.

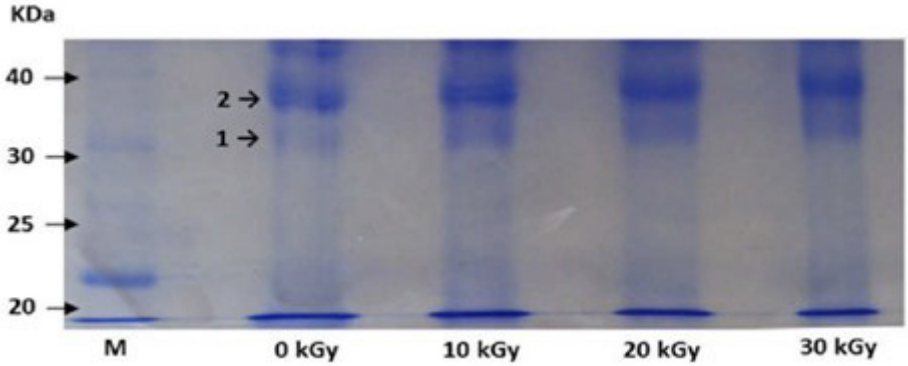

Figure 4: Yield of ricin in samples, found using chromatography

Limitations on the detection of ricin

The method suggested by (Rudolph C. Johnson, Sharon W. Lemire, 2005) suggests that a level of 20% of ricin in a urine sample is the threshold before reducing detection effectiveness. The method also depends on the impurities present to detect the presence of ricin. Ricin is often looked over in autopsies as it is hard to detect, but scientists have developed a way to detect it (Albert Einstein College of Medicine, 2009) by adding an enzyme to the adenine released by ricin. The adenine turns into ATP, which can be detected with a light emitting gene in fireflies.

Potential uses of ricin

According to studies (Rocha AJ, de Oliveira Barsottini MR, 2019), there are nine candidate genes that play a role in ricin production during the growth of a castor bean. The top 3 genes which enforced ricin production are PP2A-2, GAODG, and Elongation factor 1a. Cultivating castor beans that harbor less of these genes could lead to the reduction of ricin within castor beans, which means there is a possibility of decreasing its risk and toxicity.

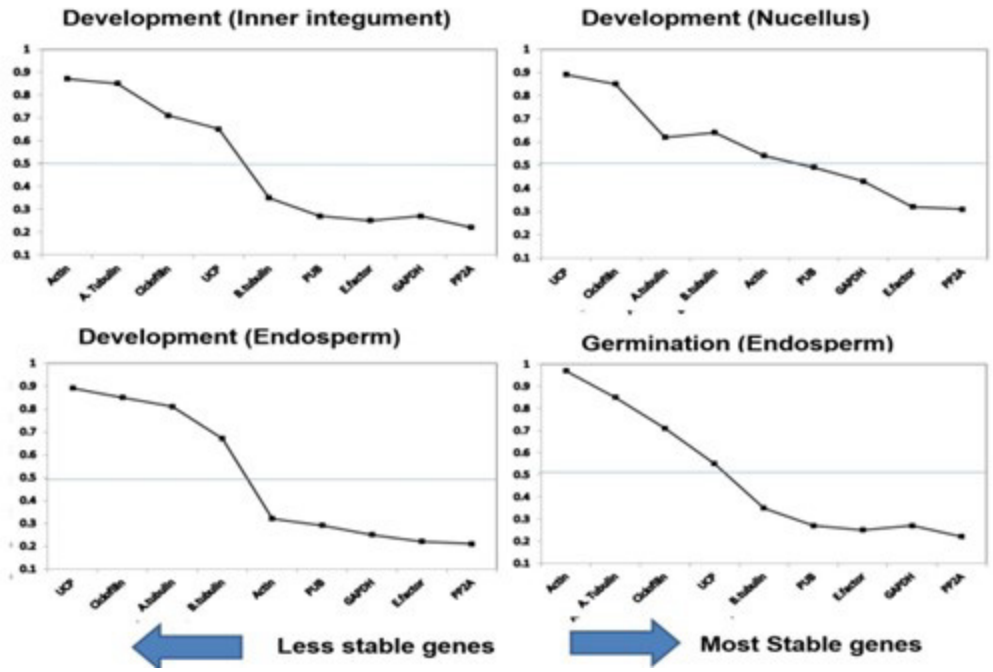

Figure 5: The development of ricin for each gene in specific areas of the castor seed

Ricin has also been reported to be a possible therapeutic agent for various cancers. Ricin's potency is high, as such, it can be used for melanoma treatment. Ricin inhibits tumorigenesis of melanoma cells, 1 and 3 ng/ml of ricin increased apoptosis of SKMEL28 up to 1.4 and 2.1 folds, suggesting that it is a useful hypothetical drug.

Conclusion

Ricin is a carbohydrate-binding protein produced from the seeds of the castor oil plant, also known as Ricinus communis. Ricin is an extremely potent toxin that's lethal in small doses. Ricin is found within the leftover broken-down mash of the castor bean. The ricin can be separated from the oil and the solvents using solvents, high pressures, and heat. The damage caused by ricin is irreversible as it causes direct cell death (it inactivates the ability of the cell to produce the necessary proteins to sustain its life). Ricin is the perfect assassination poison, as it Is easy to look over during autopsies, and even the smallest doses can be lethal, even down to 5 micrograms per Kg of a healthy adult. A singular ricin particle can kill an entire cell, a small dose of ricin is 1500 cells per minute. Ricin poisoning has no specific symptoms. There are varying ranges of intensity when it comes to specific symptoms, this applies to similar toxins and pulmonary diseases alike. The intensity is dependent on the subject, dose, and area of delivery. Hence, this makes it harder for ricin to be traced as a root cause. Ricin has been found to be a therapeutic agent for various cancers, and the castor beans, which have fewer ricin-producing genes, are being cultivated. Castor beans produce castor oil used for other purposes, including laxatives, fungal treatment, and skin treatment. Having a castor bean with little to no ricin would be beneficial in decreasing the toxicity of the castor bean. The use of ricin for cancer treatment is still being developed, but if it becomes an actual medication, the threat of tumours could be reduced.

Acknowledgments

I would like to thank my advisor for his valuable insight on this topic.

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