



## Review Paper

# Talking about beans, banes and biocrimes: colour, concentration and India's outlook on Ricin

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Available online at: [www.isca.in](http://www.isca.in), [www.isca.me](http://www.isca.me)

Received 31<sup>st</sup> December 2018, revised 10<sup>th</sup> March 2019, accepted 28<sup>th</sup> March 2019

## Abstract

*This study examines the details of the extraction procedure, analysis and the use of the biochemical compound Ricin from the seeds of the plant *Ricinus communis* (Castor). It is a very lethal chemical compound, a carbohydrate-binding protein called as lectin which can easily kill a human being within 2-5 days of exposure to it. The ricin extracted in the laboratory was not so pure to kill any lives around it and was safely disposed off after analysing its colour and concentration. A total of 48 incidents involving ricin from 1914-2018 have been reported. India is also under constant threat of a probable ricin attack with an earlier threat from Al-Qaeda and being the major player in the market of castor oil production explains the wide spread availability of *R. communis* under plantation and in wild, which is indirectly projecting a picture of ricin production as waste in the industries annually, making it an inexpensive biological tool which doesn't harm the environment and only kills their requisite living target by making it a futuristic bio weapon to be used during silent wars.*

**Keywords:** Biological warfare, castor beans, ricin, Indian biowarfare, Indian ricin.

## Introduction

Biological Warfare or bio warfare or germ warfare, is mainly concerned with the application of biotoxins, contagious factors which may include bacterial or viral or fungal and so forth, to spread infectivity among a population, to immobilize or incapacitate them. The main aim of using such a technique is to primarily, spread terror as an act of war. The weapons used in such warfare scenarios are known as biological weapons<sup>1</sup>. And one of the examples is the toxalbumin Ricin, extricated from the medium sized hard seeds of the plant *Ricinus communis* (castor bean) which holds the property of killing a human being within 2-5 days of its intake by any means.

*Ricinus communis* is commonly known as castor bean plant or wonder tree. It is an upright shrub or miniature tree with a stature of around 30 feet. It grows up to 15 feet in the colder regions. Reddish to purple joints are observed of the stalks, leaves and hollow stem. The palmate leaves are 6 to 11 lobed with uneven saw-like edges and are also red coloured which often has a blue-grey blossom. At the apex of the shoots and axes are the clusters with the separate staminate and pistillate flowers. The ripened fruit which is oblong shaped is brown in colour. There are three planate seeds present in each seedpod which erupts out when the entire fruit matures<sup>2</sup>.

The production of ricin is simple as ABC which makes it a probable biowarfare force or bioterrorist's weapon. The Centers for Disease Control and Prevention (CDC) has an information slab termed as 'Category B agent' under which ricin is placed.

This class includes biochemical or chemicals which are fairly simple to disperse, and induce pensiveness and less fatality<sup>3</sup>. The "Convention on the Prohibition of the Development, Production, Stockpiling and Use of Chemical Weapons and on Their Destruction (CWC)" classifies as a 'Schedule 1' noxious element. The up-to-the-minute report of the "Biological and Toxin Weapons Convention Procedural Report and Rolling Text"<sup>4</sup>. In USA, the CDC Select Agents and Toxins Program also supervises the dominion or carry of ricin and/or genes ciphering utilitarian forms of the toxalbumin.

## Colour and Concentration

The castor bean seeds are very difficult to crack or peel. The seeds were soaked in a solution of Lye which is generally a strong potassium hydroxide solution to make the seed covering soft to peel. With the help of pliers, the seed coat was removed and the bean pulp was mixed with acetone. It was blended well. The pulp with acetone is made to sit for two days and the acetone is drained off. This process of draining the acetone removes the castor oil and the remaining decoction present in the bottom of the beaker is ricin extract. The extract was mixed with potassium permanganate to destroy its lethal factor before disposing off. This process was only conducted to record the original colour of the ricin extract and the amount of yield<sup>5</sup>.

A 100gm of castor bean seeds yielded a wet weight of 62.108g of ricin extract. The colour of the ricin is a white with an off-whitish shade being shown while stirring it (Figure-1). The extract is quite mushy and a slight sticky in nature. It shouldn't

be dried, otherwise the ricin will spread in the environment through its powdery nature which can be very lethal, and so it should be constantly kept wet with acetone. To avoid the fumes and direct contact, a gas mask was worn with proper clean gloves which were disposed off safely by mixing with potassium permanganate.



**Figure-1:** The ricin extracted in the lab, showing its characteristic white and off-whitish shade with acetone floating over it.

## Biochemistry, Toxicity and Clinical Symptoms

‘Toxalbumin’ can be a perfect synonym for ricin which is present in the endosperm of *R. communis*’s seeds. It has been categorised under type 2 ribosome-inactivating protein (RIP). A functionally similar A chain of type 1 ribosome-inactivating protein is present in type 2 ribosome-inactivating protein which is covalently fastened to chain B by a bond which is disulfide and catalytically inactive in nature, but serves as a mechanism for transportation of conjugated amino acid complex A-B from surface of a cell to the endoplasmic reticulum’s lumen. The ribosomes are attacked by type 2 and type 1 ribosome-inactivating proteins but solely type 2 ribosome-inactivating proteins show cytotoxicity thanks to the chain B’s properties which resemble lectin in nature. The bond being disulfide in nature should be broken reductively with the objective of showing ribosome-inactivating power<sup>6</sup>.

If indrawn, administered, or eaten, ricin can prove to be extremely dangerous clinically as it impedes production of proteins<sup>7,8</sup>. The assembling mechanism of different amino acids to form proteins are stopped which is considered to be the quintessential process for the survival of species in this world.

The peptidases are unable to digest ricin as it is resistant. The gastrointestinal pathway is susceptible to ricin if ingested and can probably cause damages to the mucosal layers of the tract<sup>9</sup>.

Relying on the route of application and/or exposure, the appearance time for medical signs may take up hours or days as the symptoms are induced due to the failure in building proteins. The kidneys, liver, adrenal glands and central nervous system shows affects within 2 to 5 days of coming in contact with ricin<sup>10</sup>. The documented noxious quantity of the seeds (in number) are as follows: cocks and ducks, 80; dogs, 11; swine, 7; oxen and horses, 6; sheep, 5; rabbit, 4 and human, 2.5-6 (up to 20)<sup>11</sup>.

The experiments conducted on mice where the lethal dose is exposed to 50% of the unmasked population (LD<sub>50</sub>) and hours required for their death have been summarized in Table-1<sup>12,13</sup>. Well informed guesses based on estimated LD<sub>50</sub> and time period fatal for men left bare to ricin either from intravenous, ingestion, subcutaneous or inhalation administration of toxin have been noted based on various mammalian experiments and accidental exposures to mankind while poor ricin absorption and semi-degradation inside the gut possibly could lead to less oral toxicity. Diffuse nephritis, severe necrosis of lymphoid, diffuse splenitis, necrosis of liver and gastrointestinal hemorrhage are the common internal body aberrations seen in the human after immense doses of ricin<sup>12,14,15</sup>.

**Table-1:** Summarized results of the experiment of LD<sub>50</sub> population of mice being exposed to lethal dose of ricin.

Required dose (µg/kg)	Time required for their death (hours)	Mode of Injection
3-5	60	Inhalation
20	85	Ingestion
5	90	Intravenous
24	100	Subcutaneous

Ingestion or injecting castor bean extract has shown variety of symptoms in man which prominently includes nausea, dizziness, severe weakness, headache, back, abdominal, and chest pain 36 hours after the injection in a young man’s suicidal poisoning case after which he developed liver failure, renal failure and a bleeding diathesis, and ceased to cardiac arrest<sup>16</sup>. In another self-poisoning case with castor bean extract, the victim showed rigors 10 hours after exposure and headache after which he developed nausea, anorexia, lymphadenopathy and sinus tachycardia<sup>17</sup>.

Another recorded set of symptoms and clinical signs includes pain and weakness, followed by severe rise in body temperature, vomiting and nausea; later develops tachycardia and swollen

lymph nodes in the site of injection; later intestinal haemorrhage, signs of hypotension, renal failure and hypovolemic shock were recorded over the next 2 days; and the subsequent third day witnessed death of the individual<sup>18</sup>.

Till date, aerosol exposure to ricin cases has not been registered in human beings. Aerosol exposures are thought to induce the same internal aberrations produced in experimental studies on animals by oral or other normal exposure methods. A case study of laborers working in castor oil-processing factories<sup>19</sup> noted that an allergic syndromes have developed. The allergic conditions were marked by itchiness of the eyes, precipitous commencement of snarling-up of the nose and throat and denseness of the chest. In a review by Rauber and Heard, 751 cases of castor seed ingestion were investigated and near about 14 death cases were registered<sup>20</sup>. All the individuals show varying effects, clinical signs and symptoms, which are predominantly depended on amount of doses. The estimated fatal dose of ricin intoxication in adults with detailed pathogenesis and risk groups has been documented<sup>21</sup>. Every severe cases and incurable cases have registered same clinical days of yore: fast (less than 5 hours) onset of abhorrence, thirst, headache, abdominal pain, sore throat and vomiting succeeding by phlebotomization of the arsehole, diarrhea, spasms in abdominal muscles, expansion of the pupils, vascular collapse, anuria and fever. Generally, death occurred mainly after 72 hours in the recorded cases.

### Detection and Counter-tackling measures

Around the world, epidemiological information is still the basis of ricin poisoning which formally includes seed ingestion, or investigating a source which is clearly common to that area, or may also include several poisoning cases in a short period of time. An astringent menace and/or a surge of respiratory and/or gastrointestinal disorder is based on the clinician's methodologies and suspicion<sup>22-24</sup>. "Time-resolved fluorescence immunoassay (TRFIA)" with PCR also famously known as "Polymerase chain reaction", are being used in laboratories for qualitatively examining various ricin contaminated medicines or food articles or in determination of contamination of the environment<sup>22-25</sup>.

High performance liquid chromatography electrospray ionization mass spectrometry (HPLC-ESI-MS) is being used in the evaluation of 'ricinine', a bio-indicator of the toxalbumin present only in few selected samples<sup>24</sup>. Still, there are no designated biomarkers for detecting ricin rather a conjunction of large and small molecules, proteins, fatty acids and peptides could be used<sup>25</sup>. Weber and Schulz have described an immunologically hypersensitive technique named as "lateral flow assay (LFA)" to detect ricin quantitatively in water or food articles<sup>26</sup>. A setup of microarray-fluidic device is also being used to activate the passive pumping through the ion channels which embellishes the synthesis of cell-protein, increasing the sensitivity of detecting ricin from various beverages<sup>27</sup>.

Presently, U.S Food and Drug Administration (FDA) has not yet sanctioned any kind of vaccines or therapeutic measures or antibody therapies to combat but several trial-based-remedies have come into place which many include antibody therapies and vaccines. Vaccines like RiVax and RVEc have made their foothold as the probable ones but lacks approval of FDA. The immunogenicity and safety of RiVax have been proved in its first cycle of clinical trials, pilot trials of RiVax being adsorbed to Alhydrogel have also been stated clinically safe<sup>28</sup>. The recognition of a potential in vitro stability indicating assay as well as showing safety and immunogenicity in mice with a good shelf-life shows the efficacy of RiVax which now has been categorized under FDA's Orphan Drug Designations and Approvals<sup>29,30</sup>. Information on booster dose of RVEc measuring 50-µg being administered into four white people for first cycle of clinical trials have shown considerable success<sup>31</sup>. Some traditional detoxification methods of ricin includes treatment with hydrogen peroxide, half an hour treatment with moist heat at 200-220°C, treatment with potassium permanganate and iodine treatments are effective in destruction of the fatal actions of ricin but does not affects its haemagglutinating activity<sup>32</sup>.

### Conclusion

After South Africa, India holds a wide range of castor plant varieties growing all over its sub continental region irrespective of temperature boundaries<sup>33</sup>. The roadside invasion of the species pose a major threat to native biodiversity as well as giving a free hand to collect easy castor seeds. India has been a leading and dominant player in the industry of castor oil production around the world with around 73% of its contribution to overall world's generation which near about calculates to 150 crore kilograms of annual production<sup>34</sup>. These numbers certainly suggest easy availability of castor bean present in the Indian market and projects a humungous amount of ricin being discarded as waste products in the industries.

There are no reports of any vaccine or antibodies development programme against ricin on the cards for India's research organisations. India only has two government backed organisations, Defense Research and Development Organization (DRDO) and Defense Research and Development Establishment (DRDE) that focuses in biochemical technology, pharmacology, toxicology and the developing antibodies against different viral and bacterial threats. Disease menaces such as viral haemorrhage fever, plague, botulism, cholera, smallpox, anthrax, and brucellosis are being researched and development of probable bio-defensive works are in progress in both the centres<sup>35</sup>. The threats to use ricin in Kashmir by militants of Al-Qaeda<sup>36</sup> provides a picture of probable future ricin attacks in the country. Traditionally, some villagers have been using the method of castor poisoning to kill cattle of their rivals and also using metal nail laced with castor bean extracts to inject hard skinned cattle<sup>33</sup>.

The world has till date witnessed 48 cases of ricin in bio warfare, bio crimes and bioterrorism fields (Table-2)<sup>36-66</sup>. This

huge number of events clearly indicates that anyone who has a sound knowledge of the general extraction and purification methods of any organic compound can exploit their knowledge to extract ricin and use it in a negative way. Slower than the microbial toxins but as bacterial toxins require huge investments for extraction, it makes ricin as a first choice for any amateur or professional to kill in masses. Ricin has been considered more poisonous than a cobra's venom. These bio-apocalyptic programmes and incidents provoked by advancement in

technological front are being used for extermination, and in near future, more anthropogenic bio-apocalyptic events can be a driving factor for making surface of the earth dilapidated.

Quoting President of British Association for Advancement of Science in 2001, Sir William Stewart- "There are those who say the First World War was chemical, the Second World War was nuclear, and that the third world war, God forbid will be biological"<sup>67</sup>.

**Table-2:** Summarization of all the biowarfare programmes, bioterrorism threats and biocrimes involving the use of ricin from 1914-2018<sup>36-66</sup>.

Year, Country	Programmes/Incidents
1914, USA	U.S explored weaponizing ricin during the World War I, failed due to thermal instability of it.
1939, USA	U.S and Canada explored ricin as cluster bombs for World War II, but failed.
1942, USA	National Defense Research Committee reopened exploration ricin as an attacking toxin.
1944, USA	Under the name of Agent W, USA conducted tests of weaponized ricin.
1978, UK	Stabbing of Bulgarian writer Georgi Markov with ricin laden umbrella tip, a US supported terrorism.
1981, USA	Boris Korczak who was a CIA officer, was fired with ricin-ladenbullet in Virginia.
1982, USA	A lawyer, William A. Chanslor tried to kill his wife by ricin intoxicification.
1985, USA	A 19 year old boy was pronounced guilty for soliciting murder and was also charged for plotting to kill his father with ricin.
1991, USA	Two right wing extremists from Minnesota Patriot's Council convicted for possessing ricin.
1995, USA	An Arkansas man, Thomas Lavy was charged as he possessed 130g of ricin.
1995, USA	Debora Green food poisoned her husband, Michael Farrar with castor beans.
1997, USA	0.67g of ricin was ceased from Thomas Leahy, believed to have attempted to load a razor blade with it.
1998, USA	During an investigation of four members of the North American Militia a video tape describing the method to make ricin was found.
1998, USA	Dwayne Kuehl tried to use ricin against a city official and was convicted for it.
1999, USA	A Tampa man, James Kenneth Gluck tried to murder court judges of Colorado's Jefferson County Justice Centre using ricin.
2002, USA	Kenneth Olsen was charged for possessing 1g of ricin.
2002, UK	Six suspected terrorists were detained for serving as a "ricin laboratory" at Manchester.
2002, Iraq	Ansar al-Islam, a Sunni Militant group, was reportedly testing aerosolized ricin on animals.
2003, India	Indian securities fears the use of ricin by the terrorist outfit, Al-Qaeda.
2003, UK	Anti-terrorist police arrested six suspected people of Al-Qaida from Algeria for plotting a ricin attack on London Underground.
2003, USA	Ricin contaminated letters were procured from USA Senator Bill Frist's mailroom.

Year, Country	Programmes/Incidents
2003, UK	Six suspected members of "Chechan Network" were charged to possess castor beans, ricin purification equipments and traces of ricin.
2003,USA	Letters laced with poor ricin addressed to Department of Transportation which were intercepted at a mailroom in Greenville, South Carolina.
2003, USA	Letters covered with low potent ricin powder were mailed to White House, postmarked as Chattanooga, Tennessee.
2004, USA	Traces of ricin were found on letters addressed to Senate Majority Leader Bill Frist.
2005, USA	Florida man was detained by FBI for storing ricin and other harmful chemical products at his house.
2006, USA	Denny Ray Hughes was imprisoned for 2648 days in an attempt to make ricin.
2007, Ireland	Ricin was smuggled from U.S into Limerck Prison to be used in assassination plan in a contact lens case.
2008, USA	Roger Von Bergendorff, aged 57, was charged for carrying a bulk of ricin, guns and insurrectionistdoctrines.
2009, UK	A father-son duo was arrested from County Durham for producing ricin at home.
2009, USA	Seattle'sadvocates and apprentices at many homosexual bars were threatened with ricin poisoning.
2011, USA	Owner of a Coventry Township, Ohio was arrested by FBI for possession of ricin.
2011, USA	An erstwhile Agawan man was imprisoned to 5475 days in jail for acquiring ricin.
2011, UK	AsimKauser, a man with roots from Pakistan was held possessing instructions to make ricin.
2011, USA	Government discovered that terrorist groups are trying to procure castor beans to weaponize ricin.
2011, USA	A domestic militia group consisting four members were accused plotting and making over 4.5kg of ricin.
2012, Saudi Arabia	An herbal medicine containing ricin was reported which caused a ricin poisoning after ingestion.
2013, USA	Letters mailed to President of the USA Barrack Obama and USA Senator Roger Wicker were predicated to be laced with the toxalbumin, ricin.
2013, USA	Shannon Richardson, an American actress, was condemned for mailing ricin contaminated letters to Mayor Michael Bloomberg and USA President Barrack Obama.
2014, UK	An Indian origin daughter tried to get rid of her mother using ricin contaminated cola.
2015, UK	A Pakistan origin man tried to buy ricin online who was sentenced to 8 years in jail.
2017, USA	A 71-year old lady made ricin and tested it on her fellow residents
2018, USA	A South Carolina woman, Siers-Hill was charged for storing ricin and firearms in a rented storage unit.
2018,Germany	Wife of a Tunisian suspected jihadist was caught to possess "toxic substances" which turned out to be ricin.
2018, France	French Police foiled a suspected attack by Egyptian man, possibly involving ricin.
2018, USA	Utah man charged of mailing ground-up castor seeds in separate envelopes to US President Donald Trump, FBI Director Christopher Wray and Chief of US Naval Operations Admiral John Richardson.
2018, USA	A suspicious letter sent to US Senator Susan Collins contained ricin.

## Acknowledgement

The current study was financed and braced by Mini Project Scheme under Model for Arts and Science Teachers (MAST) Program for UG students under 'College of Excellence' for the year 2017-2018. The authors are thankful to the college authorities for backing up the work through the scheme. We are highly obliged to Dr. Samir S. Terdhalkar (Co-ordinator, Internal Quality Assurance Cell), Miss Sunanda S. Kate (Head of Department, Department of Botany) and the Principal of Fergusson College, Dr. R.G Pardeshi for their constant support and encouragement. The extraction methods mentioned herein was purely intended for the scientific study and have been conducted only after soughting official permission from the college authorities. During the study, not a single living being was hurt. Reproduction of the same procedures should be done with utmost care and vigilance under an expert. Some of the assertions and opinions presented herein involves personal perspectives of the authors and are unquestionably not the authoritative outlook of any government or private organisations.

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