WORLD JOURNAL OF PHARMACY AND PHARMACEUTICAL SCIENCES
SJIF Impact Factor 7.421
Volume 8, Issue 4, 70-78 Research Article ISSN 2278 – 4357

A STUDY TO EXAMINE THE FORENSIC TOXICITY OF RICIN

Bandr Fakiha*

Department of Medical Health services, Faculty of Health Sciences, Umm Al-Qura University, K.S.A.

Article Received on
31 Jan. 2019,

Revised on 21 Feb. 2019,
Accepted on 13 March 2019,

DOI: 10.20959/wjpps20194-13472

***Corresponding Author.**
Dr. Bandr Fakiha
Department of Medical Health services, Faculty of Health Sciences, Umm Al-Qura University, K.S.A.

ABSTRACT

Ricin is a protein-based toxin that comes from the castor bean plant (*Ricinus communis*) alongside another protein called the *Ricinus communis* agglutinin (RCA120). These two proteins are dangerous to human health. Liquid Chromatography (LC) and mass spectrometric (MS) assays are commonly used in the identification of ricin and in detecting the activity of ricin in samples with complex matrices. The methods make it possible to detect ricin from RCA120, which is less toxic. In this regard, the amino acid sequence of the ricin protein is determined by monitoring active ricin by MS. The research describes the application of LC/MS-based methods in the detection, differentiation, and quantification of ricin and RCA120 in laboratory samples. Overall, the LC/MS-based assays were used to successfully identify the samples with ricin or RCA120. In fact, mass spectrometry emerged as the most appropriate method for differentiating between ricin and RCA120. The results of the research led to the conclusion that LC/MS-based assays are efficient in detection, differentiation, and quantifying of ricin and RCA120 in any complex matrix.

KEYWORDS: Ricin; RCA120; Toxin; Castor Bean; Protein; Peptide; Liquid Chromatography; Mass Spectrometric.

1. INTRODUCTION

Ricin is a very toxic protein, which is obtained from the castor bean plant (*Ricinus communis*). This plant grows naturally in tropical and subtropical regions. Castor beans are easily accessible and thus, it is relatively easy to prepare ricin. This is the reason to argue that the toxin is a likely bioterrorism agent.[1] For instance, the CDC identifies ricin to constitute Category B agents, making it the only protein to fall among Schedule 1 chemicals.[2] As a

protein, ricin is made up of A and B chain sub-units.[3] The B chain lectin is responsible for binding receptors that contain galactose found on the surface of eukaryotic cells, thereby triggering the endocytosis of ricin. On the other hand, the A chain initiates the deadenylase activities responsible for triggering an irreversible depurination reaction of 28S rRNA to terminate the synthesis of proteins within the cell. Thus, the wide availability of ricin should raise concerns about possible deliberate contamination of food supplies.[4] While ingestion is not the only form of intoxication, there needs to be a significant effort in determining a highly sensitive methodology that can detect ricin in foods.

Exposure to ricin may occur either by ingestion or by inhalation of materials that contain ricin. However, in a few rare occasions, poisoning occurred through injections of ricin.[4] However, this is very unlikely considering that someone must be present to inject the victim with the material containing ricin. In the event of an attack involving the use of ricin, it is important that the toxin is detected and this may include the sampling of materials such as soil and foodstuffs, particularly in cases where foods contamination is suspected.[5] Considering the nature of this risk, there have been efforts in the past few years to come up with appropriate methods for detecting the presence of ricin in a range of substrates. This article examines the forensic toxicity of ricin, giving the methods attempted and the extent of achievement. Successful diagnosis of exposure to ricin toxin is critical towards initiating subsequent therapy.

2. MATERIALS AND METHODS

2.1 Safety

Given the toxic nature of ricin, all experiments involving the ricin or extracts from the castor bean plant should occur in a biosafety cabinet.[6] However, after the digestion process, the samples containing ricin are not considered toxic. This means that standard laboratory safety practices must be considered when handling it.

2.2 Preparing the antibody-coated beads

The researcher purchased Biotinylated Anti-Ricinus Communis Agglutinin (RCA) antibodies from Somatco in Jeddah. The biotinylated anti-lectins are used as intermediates to localize lectin receptors.[7] They were bought in 5 mg lyophilized aliquots and later restored using distilled water to 1 mg/mL. Additionally, the researcher bought MyOne T1 streptavidin-coated beads from ThermoFisher. The preparation of the beads was done according to

manufacturer's instructions. The basic principle of using magnetic beads is that they have an immobilized affinity to the isolated structure of the target compound.[7]

2.3 Recovering Ricin and RCA120

The researcher purchased 5 mg/mL purified solutions of ricin and RCA120 agglutinin from Somatco. The matrix sample containing toxin and agglutinin was incubated for 30 min after which the magnetic capture beads were used to recover the components. The researcher removed the capture beads from the matrix after 1 hour and washed them using 1 mL of 0.05% PBST, 1 mL of 0.01% PBST, 0.5 mL of PBS, and distilled water consecutively.

2.4 Castor Bean Extraction

Castor beans with approximately the same weight were purchased from M.A.B.M. Trading Est. To facilitate the extraction process, the researcher grinded the whole beans using a pestle tissue grinder and put the mash in the sample matrix. The researcher let the mixture to incubate for 18 h and later centrifuged it to settle large particles. The antibody-coated beads were then used to extract 0.5 mL aliquot of the supernatant, which was analyzed using the relevant methods.

2.5 Protein Digest

Protein digest process involved the use of RapiGest SF that enhances enzymatic digestion of proteins within the solution. The researcher purchased Rapigest SF from Somatco in quantities of 1 mg lyophilized aliquots. This was then converted to 0.1% using 100 mM ammonium bicarbonate. The researcher carried out protein digest experiments by buffer-exchanging magnetic beads containing ricin into the Rapigest SF solution.

2.6 Preparation of Peptide

The researcher chose ricin quantification peptides combining the A and B chains. This also facilitated the distinguishing of ricin from RCA120. However, the researcher avoided peptides that are glycosylated and those that contain cysteine, methionine, or tryptophan. The selected peptides were then synthesized.

2.7 Liquid Chromatography

Liquid chromatography facilitates the separation of a sample matrix into its individual components.[8] This separation is triggered by various interactions of the matrix sample in

stationary and mobile phases. The researcher used an Agilent 1200 capillary pump to perform the chromatographic separation. Honeywell Burdick and Jackson developed all the solvents.

2.8 Mass Spectrometric Quantification

Mass spectrometer quantification method selectively and sensitively detects and assigns a signal to a particular chemical entity, even when the chemical is present in low concentrations within the sample matrix.[1] The researcher used the LTQ module to perform mass spectrometric quantification. The samples were put in the LTQ through electrospray ionization in positive ion mode. The process recovered all the productions within a mass not exceeding the low-mass cutoff during Collision-induced dissociation (CID).

2.9 Activity Assay

Despite the existence of several methods for detecting ricin protein, the majority of the assays cannot distinguish between active and inactive ricin. The objective of the research was to develop a sensitive and selective method with the potential of totally analyzing ricin. This should take into consideration biological activities as well as structural components. The LC/MS-based methods use three layers of selectivity to detect the presence of resin and biological activities in the same sample matrix. The process involved the isolation of ricin from the laboratory samples using antibody-coated beads. The immune-purified ricin was then digested and analyzed through the LC/ MS methods to identify the toxin.

3. RESULTS

In the activity assay, the presence of ricin substrate was detected in the matrices that were spiked with 15 pmol ricin. However, this was not the case with similar pure samples. The LC/MS-based methods identified ricin peptides and further distinguished between ricin and RCA120 less toxic homolog. The greatest challenge with the detection of ricin was differentiating between the toxic and less toxic forms.[3] However, the fact that the LC/MS-based methods are able to discriminate these forms, the researcher made conclusions on the risk factors associated with each sample, even though[9] argues that detecting one unique peptide is enough to conclude that ricin is present in a mixture. According to.[3], including four more peptides as confirmation ions do not make the identification ambiguous. The additional four peptides help with distinguishing ricin from RCA120 and confirming the presence of A and B chains. The quantification process considered four tryptic peptides, with T7 peptide empirically yielding doubly charged ion and resulting in the best sensitivity. T10, T11, and T18 peptides, which were also unique to ricin produced quantitative results that

were similar to T7. Using T7 as the quantification peptide saw the low limit of detection (LOD) for ricin toxin digest be placed at 1 fmol protein.

3.1 Differentiating Ricin and RCA120

Ricin and the less toxic agglutinin protein RCA120 share more than 85% sequence homology.[10] Also, there are no known antibodies with the potential of preferentially binding to Ricin over RCA120.[9] While mass spectrometry method easily distinguishes the two proteins based on their protein sequence, quantitative measurements that use LC/MS method are limited to a few tryptic peptides. As mentioned earlier, T7 peptide from ricin distinguishes ricin from RCA120 using amino acid sequence.[11] In the case of the LC/MS experiment, the quantity of ricin obtained from the various matrices was constant at 7%, regardless of the variances in the quantity of RCA120. This is an indication that that only ricin was being quantified and that the quantity of RCA120 did not form part of the perceived amount of protein.

3.2 Castor Bean Contamination

The experiment involved the evaluation of four sample matrices containing crushed castor beans for ricin content after incubation using LC/MS method. Considering the weight of beans used, castor beans are 1.5% toxic by weight.[12] The researcher, therefore, estimated the maximum amount of ricin in the castor bean to be 10 mg, above the 1.5% range. This is because the limit of ricin recovery for the LC/MS method is high at approximately 13 pmol/ mL.[4] However, to ensure that the quantity of ricin recovered fell within the established range, the researcher split the tryptic samples into several dilute aliquots before the LC/MS analysis. After the dilution, the matrices were taken through immune-purification, protein digest, and using LC/MS, analysis processes. The researcher then evaluated the amount of ricin recovered from sample matrix using the ricin T7, T10, T11, and T18 peptides. Even though ricin was recovered from all the sample matrices with the castor bean extract, the amount of ricin varied from the first to the last sample matrix. Because the same sample of extracted ricin was used in all sample matrices, factors like pH level or matrix complexity had minimal impact on ricin recovery. However[9], noted that during the incubation of castor bean mash with the sample matrices the ricin may have denatured or bound to components like lactose, thereby accounting for the varying amount of ricin.

4. DISCUSSION

The majority of the methods used in forensic toxicology of ricin fall into three categories.[13] The first category of methods uses immunogenic interactions to detect the presence of ricin. An example of such methods is the enzyme-linked immune-sorbent assay. Another group of methods exploits the enzymatic activity of ricin.[14] Finally, the last group of methods detect castor bean DNA based on the assumption that they would contain ricin toxin as well, for example, the polymerase chain reaction. The methods are generally fast with a low limit of detection.[13] Besides, they do not require expensive instruments. However, none of the methods in the three categories represents a comprehensive assay. Even though the polymerase chain reaction is sensitive and quantitative, it indirectly tests for the presence of the ricin toxin. On the other hand, enzymatic activity-dependent measurements can be inefficient when the sample contains other toxic proteins.

Nearly all bioscience and biotechnology branches isolate, separate and purify proteins and peptides, among other specific molecules. The science and technology of separation is, therefore, a very important area that requires developments in bio-related research.[15] These create the need for the new and advanced separation techniques with the capability of treating dilute solutions that have minimal amounts of target proteins in the midst of large amounts of accompanying compounds or particulate matter, are necessary. The fact that the research used peptides corresponding to ricin and RCA120 in one analysis is advantageous over other ricin detection methods. In this regard, the LC/MS method allows for ricin to be quantified independently or alongside RCA120 depending on the quantification peptide selected.

4.1 Targeted LC/MS Approach

The reason behind using the LC/MS method was increasing the selectivity and sensitivity of the peptide quantification process.[16] acknowledge the use of mass spectrometry in targeted approaches for detecting particular peptides. The method involves using ion trap mass spectrometers for monitoring transitions the peptides dissociate from parent peptide ions. This makes the analysis of ricin peptides more precise compared to the methods described by.[13] The LC/MS method achieved the quantification of ricin at a lower limit of detection which would not have been possible with other methods. This finding is supported by the research conducted by[11], where the method was used to analyze ricin spiked into complex matrices. With a focus on A and B chain peptides that occur in ricin, the LC/MS method achieved a 0.64 ng/mL low limit of detection.

4.2 Importance of Ricin Activity Measurements

Public health investigators should be in the best position to detect protein-based toxins to enable them to react appropriately to potential natural contamination. Today, many different analytical methods are available to facilitate this analysis.[17] However, mass spectrometric techniques have proven to offer direct evidence regarding the structure of molecules by determining the amino acid sequence, measuring the molecular mass, and identifying possible modifications.[18] This may help determine whether protein toxins are behind certain diseases. In this regard, the LC/MS method identities and quantifies protein toxin to determine whether it is enzymatically active.[5] The enzymatic activities of ricin involve depurination of adenosine capable of halting protein synthesis in the human body. Research by[7] shows that mass spectrometry has the potential to monitor the enzymatic activity of ricin's A chain. However, ricin has other ribosome inactivating proteins like RCA120 that present similar enzymatic activities to ricin. This makes s LC/MS an appropriate method without necessarily requiring the supplementation of another method to address specificity. Even though[19] find the potential of ricin to be denatured in food samples, the mass spectrometry method can be used to monitor any decrease in mass of the RNA, therefore facilitating the accurate identification of possible enzymatic activities within the A chain of ricin. The LC/MS method remains to be the best for analyzing enzymatic activities of ricin as long as the specificity limitations of the assay are elaborate.

5. CONCLUSION

This research explores the potential of LC/MS-based methods to detect the presence of ricin in laboratory samples. The research sheds light on the selectivity and specificity for ricin by immunoaffinity capturing of ricin proteins, their examination, and quantification. Analysis using liquid chromatography and mass spectrometry comes with many advantages over other methods. First, the methods offer a direct measurement of ricin. The method is also not influenced by interfering proteins and highly sensitive and selective. Furthermore, the LC/MS method achieves quantification with high precision regardless of the complexity of the matrices. While the LTQ module can distinguish ricin from other proteins, the process of immune-purification was used for maintaining a low detection limit and ensures the availability of the second layer of specificity considering the fact that antibodies can selectively enrich ricin within the matrices. Finally, the choice of tryptic peptides for the forensic toxicity of ricin allows for independent quantification of ricin from RCA120. This ensures that RCA120 proteins are not quantified.

ACKNOWLEDGMENT

I would like to acknowledge my university department for being supportive when conducting the research. Further, I am grateful to the University for providing their database and resources for use in the research.

REFERENCES

1. E. B. Mubofu, "Castor oil as a potential renewable resource for the production of functional materials," *Sustainable Chemical Processes,* 2016; 4(1): 11.
2. N. Gupta, R. Noel, A. Goudet, K. Hinsinger, A. Michau, V. Pons and Y. Sakurai, "Inhibitors of retrograde trafficking active against ricin and Shiga toxins also protect cells from several viruses, Leishmania and Chlamydiales," *Chemico-biological interactions,* 2017; 267: 96-103.
3. D. Stern, D. Pauly, M. Zydek, C. Müller, M. A. Avondet, S. Worbs and B. G. Dorner, "Simultaneous differentiation and quantification of ricin and agglutinin by an antibody-sandwich surface plasmon resonance sensor," *Biosensors and bioelectronics,* 2016; 78, 111-117.
4. W. P. Bozza, W. H. Tolleson, L. A. R. Rosado and B. Zhang, "Ricin detection: Tracking active toxin," *Biotechnology advances,* 2015; 33(1): 117-123.
5. M. Diac, M. C. Matei, C. Manea, C. Schiopu, D. B. Iliescu, C. Furnica and A. Knieling, "Intoxication with Ricin-Biochemical Weapon," *Revista De Chimie,* 2017; 68(6): 1427-1430.
6. R. A. Bartholomew, R. M. Ozanich, J. S. Arce, H. E. Engelmann, A. Heredia-Langner, B. A. Hofstad and C. J. Bruckner-Lea, "Evaluation of immunoassays and general biological indicator tests for field screening of Bacillus anthracis and ricin," *Health security,* 2017; 15(1): 81-96.
7. S. Kalb, D. Schieltz, F. Becher, C. Astot, S. Å. Fredriksson and J. Barr, "Recommended mass spectrometry-based strategies to identify ricin-containing samples," *Toxins,* 2015; 7(12): 4881-4894.
8. V. Braun, V. F. Taranchenko, L. A. Tikhomirov, A. P. Grechukhin and I. V. Rybal'chenko, "Detection of Ricin in Plant Extracts and Soil Using Liquid Chromatography–High-Resolution Mass Spectrometry," *Journal of analytical chemistry,* 2018; 73(8): 786-795.

9. S. C. McGrath, D. M. Schieltz, L. G. McWilliams, J. L. Pirkle and J. R. Barr, "Detection and quantification of ricin in beverages using isotope dilution tandem mass spectrometry," *Analytical chemistry*, 2011; 83(8): 2897-2905.
10. D. M. Schieltz, L. G. McWilliams, Z. Kuklenyik, S. M. Prezioso, A. J. Carter, Y. M. Williamson and J. R. Barr, "Quantification of ricin, RCA and comparison of enzymatic activity in 18 Ricinus communis cultivars by isotope dilution mass spectrometry," *Toxicon*, 2015; 95: 72-83.
11. S. Worbs, M. Skiba, M. Söderström, M. L. Rapinoja, R. Zeleny, H. Russmann and B. Dorner, "Characterization of ricin and R. communis agglutinin reference materials," *Toxins*, 2015; 7(12): 4906-4934.
12. T. A. McKeon, D. Auld, D. L. Brandon, S. Leviatov and X. He, "Toxin content of commercial castor cultivars," *Journal of the American Oil Chemists' Society*, 2014; 91(9): 1515-1519.
13. Tevell Åberg, K. Björnstad and M. Hedeland, "Mass spectrometric detection of protein-based toxins," *Biosecurity and bioterrorism: biodefense strategy, practice, and science*, 2013; 11(S1): S215-S226.
14. M. H. Avelar, D. M. Cassimiro, K. C. Santos, R. C. Domingues, H. F. de Castro and A. A. Mendes, "Hydrolysis of vegetable oils catalyzed by lipase extract powder from dormant castor bean seeds," *Industrial crops and products*, 2013; 44: 452-458.
15. X. Liu and X. Zhang, "Aptamer-based technology for food analysis," *Applied biochemistry and biotechnology*, 2015; 175(1): 603-624.
16. S. A. Carr, S. E. Abbatiello, B. L. Ackermann, C. Borchers, B. Domon, E. W. Deutsch and D. C. Liebler, "Targeted peptide measurements in biology and medicine: best practices for mass spectrometry-based assay development using a fit-for-purpose approach. Molecular & Cellular Proteomics, 2014; 13(3): 907-917.
17. J. Dai, L. Zhao, H. Yang, H. Guo, K. Fan, H. Wang and Y. Guo, "Identification of a novel functional domain of ricin responsible for its potent toxicity," *Journal of Biological Chemistry*, 2011; 286(14): 12166-12171.
18. C. Wu, A. L. Dill, L. S. Eberlin, R. G. Cooks and D. R. Ifa, "Mass spectrometry imaging under ambient conditions," *Mass spectrometry reviews*, 2013; 32(3): 218-243.
19. M. J. Rudolph, D. J. Vance, J. Cheung, M. C. Franklin, F. Burshteyn, M. S. Cassidy and N. J. Mantis, "Crystal structures of ricin toxin's enzymatic subunit (RTA) in complex with neutralizing and non-neutralizing single-chain antibodies," *Journal of molecular biology*, 2014; 426(17): 3057-3068.