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Research article

Determination of ricin toxin in castor bean (*Ricinius communis* L.) cake samples

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Abstract: The castor plant (*Ricinius communis*), belongs to Euphorbiaceae family, whose fruits produce castor oil, but as a by-product produce ricin, a potent toxic protein, which makes handling dangerous, in addition to being considered as a biological warfare agent. The development of simple methods that detect, in a fast way, the presence of ricin toxin (RT), allows to incorporate the detoxified castor bean cakes as a nutritional supplement of animal feed. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was assayed for detecting the presence of ricin in samples of residual cakes detoxified, treated by autoclave and chemical treatment using $Ca(OH)_2$. Results confirmed the efficiency of chemical treatment, stated by biochemical patterns of proteins using this SDS-PAGE method.
Keywords: Ricin - Castor bean cake - SDS-PAGE - Chemical treatment.

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INTRODUCTION

Ricin is an albumin-like protein, with a heterodimeric structure, belonging to the type II family of ribosome-inactivating proteins (RIPs) with a molecular weight of 66 kDa. It is composed by a ribosome-inhibiting enzyme (32 kDa, A chain or RTA), bound through from a disulfide bond to a galactose/N-acetyl glucosamine-binding lectin (34 kDa, B chain or RTB) (Balali-Mood & Moshiri 2015). It is probably the most famous RIP-II, which is the only biotoxin listed in the International Convention for the Prohibition of Chemical Weapons and the International Convention for the Prohibition of Biological and Toxin Weapons (Zhu *et al*. 2018, Polito *et al*. 2019). The median lethal dose (LD_{50}) of ricin for mice is 3–5 µg kg^{-1} by intravenous injection, and for humans is 1–20 mg kg^{-1}, equivalent to 3–12 castor beans (Audi *et al*. 2005). No specific antidotes have been available in clinical applications till now (Yang *et al*. 2023).

Ricin is a potent cell toxin from the bean of the castor bean plant (*Ricinus communis* L.). Ricin toxin (RT) intoxication depends on the route of administration. Intramuscular injection causes severe localized pain and necrosis of regional lymph nodes. Inhalation induces respiratory distress with pulmonary and airway lesions. Oral ingestion causes gastrointestinal (GI) hemorrhage and liver, spleen, and kidney necrosis (Audi *et al*. 2005). Sufficient dosage induces necrosis at the injection site, as well as severe local lymphoid necrosis, GI hemorrhage, diffuse nephritis and splenitis, and liver necrosis (Audi *et al*. 2005).

Various methods for the detection of RT in animal and plant tissues, and fluids have been assayed. Liquid chromatography/mass spectrometry (LC/MS) and matrix assisted laser desorption/ionization time-of-flight (MALDI-TOF) MS methods were developed for the presumptive identification of ricin toxin and the alkaloid marker ricinine from crude plant materials (Darby *et al*. 2001). A sensitive and rapid method, antibody-independent assay for the identification of ricin in body fluids using mass spectrometry has been reported

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(Feldberg *et al*. 2021). Enzyme-linked immunosorbent assays (ELISA) have become in a very reliable method, due to the high sensitivity, provided by the use of monoclonal antibodies, which ensures the reproducibility of the assay (Peraile-Muñoz *et al*. 2017). Detection of ricin genomic DNA by polymerase chain reaction (PCR) is also a powerful and reliable method for the detection and characterization of ricin (Sook-Kyung *et al*. 2006). Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) has been suggested and assayed, but no standard and approved RT detection method currently exists.

The objective of this work was the development of a biochemical assay based on SDS-PAGE, for rapid detection of ricin in samples of residual cakes obtained for nutritional supplement for animal feed.

MATERIALS AND METHODS

The oil extraction process consisted of separating the fatty part of the castor seeds from the other components. The dry seed that met the quality criteria was subjected to the pressing process in a hydraulic press. A pressure range of 80 bar was applied. The oily mass obtained falls into a container after being previously filtered (filtration I) through a mesh or strainer. The remaining solid or cake is subjected to a second pressing and subsequently recovered as a by-product.

Castor cake detoxification

Two methods were tested. The physical method used was autoclave sterilization (1 atm, 15 psi. for 60 min). The chemical detoxification of the castor cake was carried out using a $Ca(OH)_2$ solution (0.1 g per ml of water), as described by De Oliveira *et al*. (2007), that is to say that for 10 liters of the solution, 16 kg of cake were added. It was mixed and left to rest for 24 hours and later it was dried by exposure to the sun.

Protein diagnostic

Protein diagnostic was performed employing Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE) (Manns 2001), in denaturing conditions.

In an eppendorf tube, one ml of 1% NaCl extraction buffer was added to 0.1 grams of residual cake, and centrifuged at 10,000 rpm for 20 minutes at 25°C. The supernatant (crude protein extract) was removed. For electrophoresis, to 10 µl of this extract, 20 µl of SDS buffer (60 mM Tris–HCL, pH 6.8), 100 ml l^{-1} of glycerol, 20 g l^{-1} of sodium dodecyl sulfate (SDS), 0.25 g l^{-1} of L of bromophenol blue and 0.25 g l^{-1} of β-mercaptoethanol) were added. The mixture was subjected to 100°C for 5 minutes after which 20 µl were taken to deposit them in each well of the gel. Initially the electrophoretic run was conducted at 50 volts until the dye band reached the surface of the separator gel, thereafter it was increased to 140 volts, where it was maintained until the dye approached the lower extremity of the gel, where the electrophoretic run was terminated. The gels were removed from the chamber and subjected to a staining solution (1.5 g Coomassie Brilliant Blue, 90 ml glacial acetic acid, 450 ml ethanol and 460 ml distilled water) for 24 hours and then distaining (100 ml of glacial acetic acid, 500 ml of ethanol and 400 ml of distilled water) for 12 hours. For the identification of ricin fractions, a molecular weight marker from 6.5 to 200 kDa (BIORAD) was used.

RESULTS AND DISCUSSION

SDS-PAGE application allowed to characterize ricin by purity and migration behavior on the acrylamide gel and has been shown in figure 1. Samples were run on 15 % SDS-Polyacrylamide gel. Lanes 1, 4 and 7 correspond to castor bean cake (0.1, 0.3 and 0.5g respectively) without treatment. A double band of around 31 kDa is observed. Lanes 2, 5, 8, corresponding with castor bean cake (0.1, 0.3 and 0.5 g respectively) treated with autoclave, did not show the expected effect on the protein band. A decrease in the presence of protein bands is observed, including the double band corresponding to the protein ricin. Nevertheless, this result is not consistent with the previously obtained by Anandan *et al*. (2005), who determined that autoclaving was the physical method that was able to completely remove ricin. This means that autoclaving treatment was not sufficiently efficient to fully detoxify the sample. At the same time, lanes 3, 6, 9, corresponding with castor bean cake (0.1, 0.3 and 0.5 g respectively), treated with $Ca(OH)_2$ evidenced the disappearance of all the protein bands with this compound. The disappearance after the $Ca(OH)_2$ treatment assayed for ricin detoxification, confirmed the effectiveness of this treatment.

In practical terms, $Ca(OH)_2$ treatment is easier and cheaper than autoclaving treatment, which requires the existence of the apparatus. At the same time, the amount for detoxifying is considerably lower using the physical method. That means, the result obtained satisfies the requirements for the practical application of this technique. We have characterized the purity and the subunit composition of ricin using SDS-PAGE.

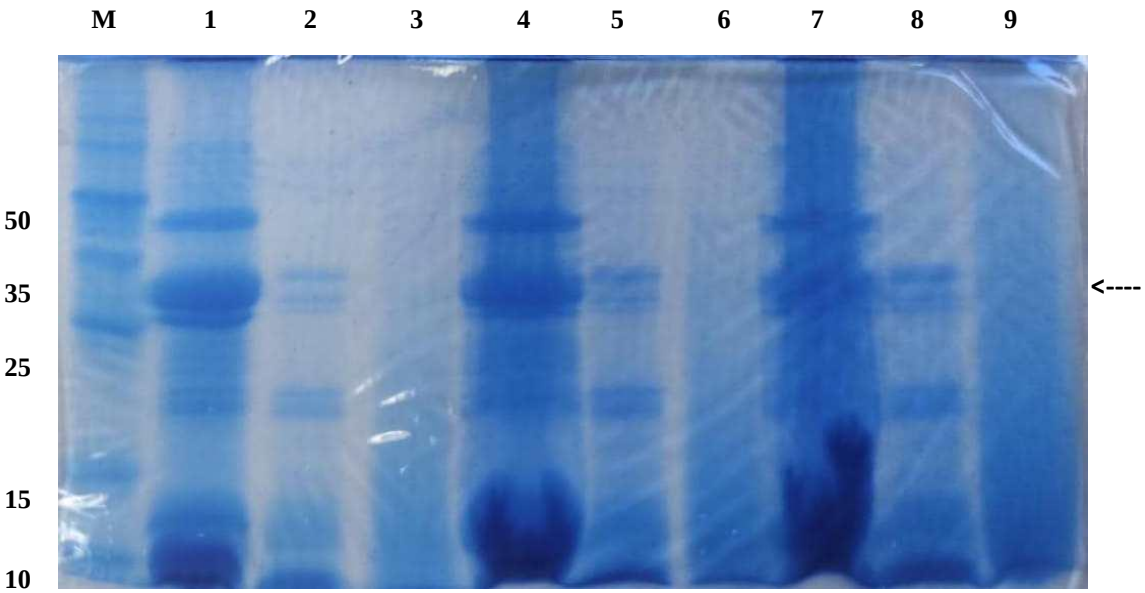

Figure 1. SDS-PAGE of ricin determination: M- Molecular weight marker; Lanes 1, 4, 7- Castor bean cake (0.1, 0.3 and 0.5g) without treatment; Lanes 2, 5, 8- Castor bean cake (0.1, 0.3 and 0.5g) treated with autoclave; Lanes 3, 6, 9- Castor bean cake (0.1, 0.3 and 0.5g) treated with $Ca(OH)_2$. [Observe the double bands corresponding to the proteins ricin and agglutinin and disappearance of all the protein bands after treatment with $Ca(OH)_2$]

Ramírez-Hernández (2017) employed SDS-PAGE method for confirmation of detoxification of castor bean cake for use as nutritional supplement of bovine livestock.

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REFERENCES

Anandan S, Kumar GKA, Ghosh J & Ramachandra KS (2005) Effect of different physical and chemical treatments on detoxification of ricin in castor cake. *Animal Feed Science and Technology* 120(1–2): 159–168.

Audi J, Belson M, Patel M, Schier J & Osterloh J (2005) Ricin poisoning: a comprehensive review. *JAMA: The Journal of the American Medical Association* 294(18): 2342–2351.

Balali-Mood M & Moshiri M (2015) Problems of clinical diagnosis and management of a deliberate biological born disease. *Journal of Bioterrorism & Biodefense* 6: e113. [DOI: 10.4172/2157-2526.1000e113]

Darby SM, Miller ML & Allen RO (2001) Forensic determination of ricin and the alkaloid marker ricinine from castor bean extracts. *Journal of Forensic Sciences* 46(5): 1033–1042.

De Oliveira AS, Oliveira MRC & Campos JMS (2007) Eficácia de diferentes métodos de destoxificação da ricina do farelo de mamona. In: *II Congresso da Rede Brasileira de Tecnologia de Biodiesel, Brasília. Anais*. CD-ROM Brasília: MCT/ABIPTI, pp. 1–6.

Feldberg L, Elhanany E, Laskar O & Schuster O (2021) Rapid, sensitive and reliable ricin identification in serum samples using LC-MS/MS. Toxins (Basel). 13(2): 79. [DOI: 10.3390/toxins13020079]

Manns JM (2001) *SDS-Polyacrylamide Gel Electrophoresis (SDS-PAGE) of proteins. Current Protocols in Microbiology*. Available from: http://dx.doi.org/10.1002/9780471729259.mca03ms22 (accessed: 11 Feb. 2023).

Peraile-Muñoz I, Lorenzo- Lozano P, Gil- García M, González- López L, Cabria- Ramos JC & Jiménez- Pérez MV (2017) Protocolo para la identificación rápida y sensible de ricina en muestras ambientales ante una alerta biológica. *Sanidad Militar* 73(3): 153–157.

Polito L, Bortolotti M, Battelli M, Calafato G & Bolognesi A (2019) Ricin: An Ancient Story for a Timeless Plant Toxin. *Toxins* 11: 324.

Ramírez-Hernández IC (2017) Potencial de uso de la torta de higuerilla (*Ricinus communis* L.) como suplemento alimenticio para la producción bovina. Tesis de MSc. Universidad Nacional de Colombia,

Facultad, Ciencias Agrarias. Medellín, Colombia

Sook-Kyung K, Hancock DK, Wang L, Cole KD & Reddy PT (2006) Methods to characterize ricin for the development of reference materials. *Journal of Research of the National Institute of Standards and Technology* 111(4): 313–324.

Yang Z, Wang C, Liu J, Xiao L, Guo L & Xie J (2023) *In silico–Ex Vitro* iteration strategy for affinity maturation of anti-ricin peptides and the SPR biosensing application. *Toxins* 15(8): 490.

Zhu F, Zhou Y, Ji Z & Chen X (2018) The plant ribosome-inactivating proteins play important roles in defense against pathogens and insect pest attacks. Front. *Plant Science* 9: 146.