Comparable LD_{50} of *Ricinus communis* extract by different routes of administration in rabbits

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Abstract

In this study dried clean seeds of castor oil were used to obtain deafening cake which dried, obtain whitish-beige, fine powder (extract- I), this extract was submitted to addition process by mixing with water, mixed thoroughly by electric blender till homogenization then filtered and obtain more clean powder (extract II). The powder of extract I and extract II was dissolved in water and used in the experiment. The results of this study showed that; LD_{50} of oral administration of extract I was 352.58 mg. while LD_{50} of intraperitoneal, or intramuscular injections of extract I was 6.11 mg. Meanwhile $LD_{50\ of}$ intraperitoneal, or intramuscular injections of extract II was 3.11 mg. Occurrence of death was within 10-24 hr post exposure in case of I.P. and I.M. injection, while in oral exposure two animals were died within 24 - 72hr. The remaining were died within 24 hr. The main signs monitored on exposed animal that died were depression, loss of appetite, comatose for 30-60 minutes, then die. Post mortem findings nearly all internal organs, liver, heart, lungs, kidney, brain, and digestive system showed signs of poisoning mainly congestion. In conclusion, there was no difference between I.P and I.M. administration of both extract I and II. The LD50 of extract II was half of that of extract I. which means that extract II was more purified.

Keywords: Rricinus communis, LD_{50}
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مقارنة الجرعة القاتلة 50 لمستخلص نبات الخروع بطرق مختلفة من التعرض في الأرانب

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الخلاصة

استخدمت في هذه الدراسة بذور نبات الخروع النظيفة الجافة، للحصول بعد الصم على كيكة، والتي جففت للحصول على مسحوق ناعم ابيض ببياض الصوف. واعتبر هذا المستخلص 1، اخضع المستخلص 1 إلى عملية إضافية بمزجه مع الماء ووضع في الخلاط الكهربائي لحين الحصول على خليط متجانس، والذي رشح بورق الترشيح ثم جفف للحصول على مسحوق المستخلص الثاني 11. أذيب كل من المستخلص 1 و11 في الماء واستخدمت في التجربة لتقدير الجرعة القاتلة 50 للخروع. تم تعريض الحيوانات للمستخلص 1 عن طريق الفم، والحقن داخل الخلب، وداخل العضل، بينما تم التعرض للمستخلص11 عن طريق الحقن داخل الخلب والعضل. أظهرت نتائج الدراسة أن الجرعة القاتلة 50 للخروع في الأرانب للمستخلص 1 عن طريق الفم كانت 352.58 ملغم. ينما عن طريق الحقن داخل الخلب، وداخل العضل فكانت 6.11 ملغم. في حين كانت الجرعة المهلكة للمستخلص 11 عن طريق الحقن داخل الخلب، وداخل العضل فكانت 3.11 ملغم. حدث الهلاك خلال 10– 24 ساعة من التعرض للحقن داخل الخلب، أو داخل العضل. بينما في التعرض عن طريق الفم هلكت الحيوانات خلال 24 ساعة، باستثناء حيوانان هلكت بين 24– 72 ساعة. شملت العلامات السريرية الرئيسية التي لوحظت على الحيوانات قبل الهلاك على الانخساف، فقدان الشهية،

لوحظ الإغماء لمدة 30–60 دقيقة، ثم الهلاك. وفي التشريح تم ملاحظة إن جميع الأعضاء (الكبد، الكلى، القلب، الدماغ، الرئتين، والجهاز الهضمي) أظهرت علامات التسمم وبالأساس الاحتقان. ويمكن الاستنتاج بان ليس هناك فرق بين الحقن داخل الخلب أو داخل العضل لكلا المستخلصين، وان الجرعة في المستخلص 11 والذي يعتبر أكثر نقاوة وتركيزا من المستخلص 1 كانت نصف الجرعة للمستخلص 1.

الكلمات المفتاحية: الجرعة القاتلة، الخروع.

Introduction

R. communis L, belong to Euphorbiaceae family; common named plant castor oil (1) Phytosterols proteins, fatty acids, coumarins, phenolic compound (2), flavonoids (3, 4), alkaloids (4), terpenoid and tocopherol- related compounds (5) have already been isolated from different parts of this plant. Ricin the toxic substance, found in the bean of the castor plant, is one of the most toxic and easily produced plant toxins. It is consisting of two polypeptide chains, A chain and B chain, linked by a disulfide bond. It is one of a group of di-chain ribosome- inactivating proteins, which are specific for the depuration of a singles adenosine in ribosomal ribonucleic acid (RNA) (6). Two ricin agglutinins and two toxins have been identified. All four lectins consist of two different polypeptide chains joined by a disulfide bond, the toxins are dimmers of an A chain and a B chain (7). Ricin, extracted from the castor bean, exerts its cytotoxicity by two separate mechanisms. The B chain (RTB) linked by a disulfide bond, and binds to the terminal galactose of cell surface glycolipids and glycoproteins. The bound toxin then undergoes endocytosis and is transported via endosomes to the Golgi apparatus and the endoplasmic reticulum. The A chain (RTA) in translocated to the cytosol, where were stops protein synthesis by inhibits protein synthesis by irreversibly inactivating eukaryotic ribosomes through removal of a single adenine residue from the 28S ribosomal RNA loop contained within the 60S subunit. This process prevents chain elongation of polypeptides and leads to cell death (8, 9). In mice, intravenously injected ricin was distributed mainly to the spleen, kidneys, heart, and liver, while intramuscularly injected ricin was found to localize in draining lymph nodes (10). (125) I-Labeled ricin injected either intravenously or intraperitoneal in mice was distributed in various tissues, accumulating in the spleen, kidneys, heart, liver and thymus. Urinary excretion of radioactive degradants, but not intact ricin, peaked 5 to 7 hr after injection and was complete within 10-20 hr. (11). The risk of toxicity from skin exposure to ricin is low, ricin may be absorbed through irritated, damaged, or injured skin or through normal skin if aided by a solvent carrier (12and 13). As a relatively large protein, ricin is unlikely to be extensively absorbed from the gastrointestinal tract. In animal studies, most orally administered ricin was found in the large intestine after 24 hours, with only limited systemic uptake (14). Poisoning of animals with *R. communis* seeds, a variability in toxicity was observed. whereas horses seem to be most sensitive, followed by geese, rodents, ruminants and chicken seem to be most resistant animals (14). The sign of toxicity developed most frequently within 6-4 h. In biochemical examination, a high packed cell volume as a sign of severe dehydration, high serum creatinine kinase and AST as well as high concentrations of serum BUN and creatinine have been observed (15). Toxicity of ricin has mostly been determined with toxin preparations containing a mixture of differently glycosylated ricin isoforms. Toxicity data might also depend on the application of different purification protocols, including acid precipitation or salt conditions, all resulting not only in different purities but also different functional activities (16).

Furthermore, a certain degree of variability in toxicity data is linked to the experimental system used, e.g., the animal species or strain used and the cell culture or in vitro assay used (17, 19). After an oral exposure, most of the ricin is found in the large intestine for 24 hrs, after ingestion, illustrating the limited systemic uptake of the protein (20). Based on mouse toxicity LD50 data, approximately 0.025% of the ingested ricin is absorbed following oral administration, but other work has shown that up to 0.27% of the ingested ricin may be absorbed (21). Once absorbed ricin is most likely distributed throughout the extracellular fluid space in the body (22). Ricin appears to be readily absorbed via the inhalation route, but dermal absorption is unlikely to occur through intact skin (23). Intravenously administered ricin distributes primarily to the spleen, kidneys, heart, and liver, and intramuscularly administered ricin distributes to draining lymph nodes (20).

Material and Methods

An amount of seeds of R. communis were collected from different shrubs distributed in Baquba city, Diyala, Iraq.

- **Extraction:** Seeds are cleaned and washed with tap water, dried. The outer husks of the seeds were manually removed, the residual wet flesh was ground into pulp. The pulp was pressed mechanically through hydraulic pressure, till remove all of the oil in the pulp. The whitish scums were mixed by the blender with petroleum ether for complete defatting of the castor oil, the mixture was filtered by filter paper and cotton tissue to separate the cake from the castor oil and petroleum ether. The cake was dried using desiccators containing NaOH and the final result was dry, whitish-beige, and fine powder (Extract I) kept, till use. (24). The extract II was obtained through additional process carried upon the fine powder of extract I, by dissolved it in water and mixed by electric blender till homogenization then filtered and dried from water. The exposed animal was monitored post exposure for a period extended to 24-72 hr. nearly all exposed animals died within 10-24 hr post exposure with the main signs of depression for 2-3 hr then comatose for 30. 60 minute and die. We follow the following formula to calculate LD_{50} (according to 25) up and down method:

  LD_{50} = xf+kd

  Where. LD_{50} = Median lethal Dose.

  xf=Last dose used in the experiment

  D=Different between doses

  K= Factor of change from the table

  O=Symbol of survival animal after 24 hours of dosing

  X=symbol of dead animal after 24 hours of dosing.

  Extract I:

  Dissolved in water, administered orally.

First rabbit was exposed to 450 mg/ kg b. wt. was died, so we give less dose to 2nd rabbit 375 mg /kg b. wt. which also died, the 3rd rabbit was exposed to 300 mg / kg. b. wt., which remain live, so the 4th rabbit was exposed to 375 mg / kg b. wt., which die, the 5th one exposed to 300 mg /kg. b. wt. remained live and lastly the 6th rabbit exposed to 375. Mg /kg b. wt. died.

Formula: XXOXOX

The last dose was 300 mg, k factor from table for above formula is (0.701), the difference between doses=75

So LD_{50} of *R. communis* toxin through oral administration in rabbits:

300 + 75 (0.701) = 352.58 mg

Extract I administered through intraperitoneal

We start with a dose of 11 mg /kg b. wt. of R. communis toxin, the 1st rabbit was died so we decreased the dose to 9.5 mg/ kg b.wt., 2nd rabbit was died, the 3rd dose was decreased to 8 mg/ kg b. wt. and rabbits dies, the next dose was 6.5 mg/ kg b. wt. and rabbits also died, next dose was 5 mg /kg b. wt. here the rabbits remain alive, so we increased dosed to 6.5 mg / kg b. wt. and rabbits died, when dose decreased to 5 mg /kg b. wt. rabbits remain alive, and lastly, we increased it to 6.5 mg /kg b.wt. and rabbit died.

So, the formula was: XXXXOXOX

Last dose 5 mg, k=0.741, d=1.5

5 + 1.5 (0.741) = 6.11 mg is the LD_{50} of *R. communis* toxin I.P.

Extract I administered through intramuscular

The doses and procedures as in intraperitoneal injection (above):

So, the formula was: XXXXOXOX

Last dose 5 mg, k=0.741, d=1.5

5 + 1.5 (0.741) = 6.11 mg is the LD_{50} of *R. communis* toxin I.M.

Extract II: through intraperitoneal injection

We start with a dose of 8 mg /kg b. wt. of R. communis toxin, the 1st rabbit was died so we decreased the dose to 6.5 mg/ kg b.wt., 2nd rabbit was died, the 3rd dose was decreased to 5 mg/ kg b. wt. and rabbits dies, the next dose was 3.5 mg/ kg b. wt. and rabbits also died, next dose was 2 mg /kg b. wt. here the rabbits remain alive, so we increased dosed to 3.5 mg/ kg b. wt. and rabbits died, when dose decreased to 2 mg/ kg b. wt. rabbits remain alive, and lastly, we increased it to 3.5 mg/ kg b.wt. and rabbit died.

The formula XXXXOXOX

Last dose 2 mg, k=0.741, d=1.5 so

2 + 1.5 (0.741) = 3.11 mg is the LD_{50} of *R. communis* toxin I.P

Extract II: through intramuscular injection

The doses and procedures as in intraperitoneal injection (above):

The formula: XXXXOXOX

Last dose 2 mg, k=0.741, d=1.5 so

2 + 1.5 (0.741) = 3.11 mg is the LD_{50} of *R. communis* toxin I.P

Results and Discussion

The results of the study showed that: LD_{50} of *R. communis* toxin, through oral administration of extract I was 352.58 mg. while, in case of Intraperitoneal or intramuscular administration was 6.11 mg. meanwhile, in case of extract II administered through I.P. or I.M. was 3.11mg. In our study, most of exposed animals that died were died within 10-24 hr post exposure in case of I.P. and I.M. injection, while in oral exposure two animals were died within 24 hr-72hr, the remaining were died within 24 hr. The main signs monitored on exposed animal that died were depression, loss of appetite, then comatose for 1-3 hours, then died. On post- mortem, nearly all internal organs, Liver, heart, lungs, kidney, brain, and digestive system showed congestion and signs of poisoning. Ricin acts in a time-and concentration-dependent manner (16, 26). There is a time delay of about 10 h. before death occurs even with very high doses applied (16). Intravenous injection of ricin into mice, the dose that produces (LD_{50}) was found to lie between 2-8 µg/ kg body weight (16, 27). (16, 19, 28, 29, 30). In rats 0.35- 0.5 µg/ kg b. wt.; guinea pigs 0.4- 0.5 µg/ kg; rabbits 0.03-

0.06 µg/ kg and dogs 1.65- 1.75 µg/ kg. Were reported (16). Somewhat more divergent amounts between 2.4 and 36 µg/ kg were needed to produce death in 50% of mice after intraperitoneal injection (19, 26, 30, 31, 32). The inhalational toxicity in estimated LD_{50} was reported to be between 2.8 and 12.5 µg/ kg in different mouse strains (18; 33). LD_{50} in rat intratracheally was 5 ug/ kg.; Parenterally 336 ng/ kg.; intraperitoneally1500 ng/ kg. (34). Oral absorption is poor and absorption through intact skin most unlikely; the moist hazardous routes of exposure being inhalation and injection (20). There is greater than a 100- fold difference between the susceptibility of various species (14). The oral lethal dose (Oral LD_{50}) of seed material (assuming 1% to 5% ricin concentration) has been reported for the following species: chicken 14 g/ kg (140 to 170 mg of ricin/ kg) (35). Swine 1.3 g/kg (13 to 65 mg ricin/ kg) (35). Rabbit 0.9g/ kg (9 to 45 mg ricin/ kg) (36). Horse 0.1 g/ kg (1 to 5 mg ricin /kg) (23). The castor seeds contain approximately 0.2% of the alkaloid. LD_{50} values for ricinine were 340 mg/ kg for intraperitoneal and 3 g/ kg for oral incorporation (37). The toxic or lethal dose of ricin depends upon the species exposed and the route of exposure (23). There is a 100-fold variation in the lethal toxicity of ricin for various domestic and laboratory animals, per kilogram of body weight, of animals tested, the chicken and frog are least sensitive, while the horse is the most (14). Toxicity of ricin also varies with route of challenge. In laboratory mice, the approximate LD_{50} and time to death are respectively, 3 to 5 µg/ kg, 60 hours; by inhalation, 5 µg/ kg, 90 hours; by intravenous injection, 22 µg/kg, 100 hours; by intraperitoneal injection, 24 µg/ kg, 100 hours; by subcutaneous injection 20 mg/ kg, 85 hours; by intra-gastric administration. Low oral toxicity reflects poor absorption of the toxin from gastrointestinal tract. Higher toxicity by other routes may be directly related to accessibility of target- cell populations and the ubiquity of toxin receptors throughout the cells of the body, when skin tests were performed on mice, no dermal toxicity was observed at the 50 µg/ spot (38).

References

1. Ross, I. A. (2001). First ed. Medicinal Plants of the world: Chemical Constituents Traditional and Modern Medicinal Uses, 2. Humana Press, Totowa, New Jersey. 296.
2. Williamson, E. M. (2002). Major Herbs of Ayurveda, first ed. Elsevier Science, Churchil Living stone, China.
3. Byamukama, R.; Jordheim, M.; Kiremire, B. & Anderson, O. M. (2008). New anthocyamins from the stem bark of castor, Ricinus communis. Nat. Prod. Comm., 3: 1497-1500.
4. Kang, S. S.; Cordell, G. A.; Soejarto, D. D. & Fong, H. H. S. (1985). Alkaloids and flavonoids from Ricinus communis. J. Nat. Prod., 48: 155- 156.
5. Tan, Q. G.; Cai, X. H.; Du, Z. Z. & Luo, X. D. (2009). Three terpenoids and tocopherol-related compound from Ricinus communis. Helvetica Chimica Acta, 92: 2762-2768.
6. Barbieri, L.; Baltelli, M. & Stirpe, F. (1993). Ribosomes-in activating proteins from plants. Biochemica Biophysica Acta.,1154: 237-282.
7. O ‘Neil, M. J. (2006). The Merck Index- An Encyclopedia of Chemicals, Drugs, and Biologicals. White house Station, NJ.; Merck and Co. Inc: 1415.
8. Lord, M. J.; Jolliffe, N. A.; Marsden, C. J.; Cathgrereine, J.; Pateman, S. C. C.; Smith, C. D.; Snooper, A. R.; Watson, D. P. & Robert, M. L. (2003). Ricin: mechanisms of cytotoxicity. Oxicol. Rev., 22: 53-64.

9. Goldfrank, L. R. (2011). Goldfrank's Toxicologic Emergencies 9th Ed. McGraw- Hill, New York, N. Y., L 1549.
10. Gupta, R. C. (2009). Handbook of Toxicology of Chemical warfare agents. P. 340.
11. Krieger, R. (2001). Handbook of Pesticide Toxicology. 1 2nd ed. Academic Press, San Diego, California. P. 162.
12. Center for Disease Control and Prevention. (2013). Emergency Response Safety and Health Database: Ricin Biotoxic. Available from as of April, P. 18.
13. Marrs, T. C.; Maynard, R. L. & Sidell, F. R. (2007). Chemical Warfare Agents. Toxicology and treatment, P. 617.
14. Balint, G. A. (1974). Ricin. The toxic protein of castor oil seeds. Toxicology, 2: 77-102.
15. Aslani, M. R.; Maleki, M.; Mohri, M.; Sharifi, K.; Najjar-Nezhad, V. & Afshari, E. (2007). Castor bean (Ricinus communis) toxicosis in a sheep flock. Toxicology, 49: 400- 406.
16. Fodstad, O.; Olsnes, S. & Pihl, A. (1976). Toxicity, distribution and elimination of the cancer ostatic lectins arbin and ricin after parenteral injection into mice. Br. J. Cancer., 34: 418- 425.
17. Poli, M. A.; Roy, C.; Huebner, K. D.; Franz, D. R. & Jaax, N. K. (2007). Ricin. In: Medical Aspects of Biological Warfare, 2nded.; Dembek, Z. F. Ed.; TMM publications: Washington, DC, USA. PP. 323-335.
18. Wannemascher, R. W. & Anderson, J. B. (2006). Inhalation Ricin: aerosol Procedures, Animal Toxicology, and Therapy. In Inhalation Toxicology; Salem H.; Katz, S.A., Eds.; CRC Press Taylor and Francius Group: Boca Raton, FL, USA. PP. 973- 982.
19. Foxwell, B. M.; Detre, S. I.; Donovan, T. A. & Thorpe, P. E. (1985). The use of anti – ricin antibodies to protect mice intoxicated with ricin. Toxicology, 34: 79- 88.
20. Bradberry, S. M.; Dickers, K. J.; Rice, P.; Griffiths, G. D. & Vale, J. A. (2003). Ricin poisoning. Toxicol. Rev., 22: 65.
21. Olsnes, S. & Kozlov, J. V. (2001). Ricin. Toxicon (Oxford), 39: 1723.
22. Shelton, G. D. (2002). Myasthenia gravis and disorders of neuromuscular transmission. Vet. Clin. North Am. Small Anim. Pract., 32(1):189-206.
23. Greenfield, R. A.; Brown, B. R.; Hutchins, J. B.; Iandolo, J. J.; Jackson, R.; Slater, L. N. & Bronze, M. S. (2002). Microbiological, Biological and chemical weapons of warfare and terrorism. Am. J. Med. Sci., 32: 323-326.
24. Al-Tahan, F. J. & Al-Shaha, O. M. S. (1990). A primary study on castor beans cultivated in Iraq and it's content of the toxic substance. Ricin. The Proceeding of the 2ndTechinal Education Conference, Baghdad, Iraq, PP. 227-240.
25. Dixon, W. J. (1980). Efficient analysis and experimented observations. Ann. Rev. Pharmal. Toxical., 20:441-62.
26. Zhan, J. & Zhou, P. A. (2003). Simplified method to evaluate the acute toxicity of ricin and ricinus agglutinin. Toxicology, 186: 119-123.
27. Olsnes, S.; Saltvedt, E. & Pihl, A. (1974). Isolation and comparison of galactose-binding lectins from Abruspre catorious and Ricinus communis. J. Biol. Chem., 249: 803-810.
28. He, X.; McMahon, S.; Henderson, T. D. II.; Griffey, S. M. & Cheng, L.W. (2010). Ricin toxicokinetics and its sensitive detection in mouse serial feces using immune-PCR. Plos one, 5 (9):e12858.

29. Jang, H. Y. & Kim, J. H. (1993). Isolation and biochemical properties of ricin from Ricinus communis. Korean Biochem. J., 26: 98-104.
30. Lin, J. Y. & Liu, S. Y. (1986). Studies on the antitumor lectins isolated from the seeds of Ricinus communis (castorbean). Toxicology, 24: 757-765.
31. Roy, C. J.; Hsale, M.; Hartings, J. M.; Pitt, L. & Duniho, S. (2003). Impact of inhalation exposure modality and particle size on the respiratory deposition of ricin in BALB. mice. Inhal. Toxicolo., 15: 619-638.
32. Lewis, R. J. Sr. (2004). Sax's Dangerous Properties of Industrial Materials. 11thEdition. Wiley -Inter science, Wiley and Sons, Inc Hoboken, NJ., P. 3160.
33. Garland, T. & Bailey, E. M. (2006). Toxins of concern to animals and people. Rev. Sci. Tech. off Int. Epiz, 25 (1): 341-351.
34. Ma, L.; Chia-Hsuh, H.; Patterson, E.; Thadani, U. & Robinson, C. P. (1996). Ricin depresses cardiac function in the rabbit heart. Toxicol. Appl. Pharmacol., 138: 72.
35. Ferraz, A. C.; Angelucci, M. E. M; Da Costa, M. L.; Batista, I. R.; de Oliveria, B. H. & da Cunha, C. (1999). Pharmacological evaluation of ricinine, a central nervous system stimulant isolated from Ricinus communis. Pharmacol. Biochem Behav., 63: 367- 375.
36. Wannemaker, R. Assistant Division Chief. (1994). Toxicology Division, US Army Medical Research Institute of Infectious Diseases, Fort Detrick, MD. Personal communication.
37. Rantuccio, F.; Sinidsi, D.; Scardigno, A. & Coviello, C. (1981). Histological changes in rabbits after application of medicaments and cosmetic bases. II. Contact Dermatitis, 7: 94-97.
38. Migally, N. (1979). Effect of Castor oil and benzoyl benzoate used as a vehicle for anti-androgens ion the adrenal cortex. Arch. Androl., 2: 365-369.