International Journal of Pharmacology

ISSN 1811-7775

science alert

ansinet
Asian Network for Scientific Information

OPEN ACCESS International Journal of Pharmacology

ISSN 1811-7775
DOI: 10.3923/ijp.2024.54.61

Research Article
Ricinine Distribution in Blood and Tissue After Oral Intoxication-The Data Augmentation Approach

[1]Radosav Radosavkić, [1]Maja Đurendić Brenesel, [2]Vladimir Pilija, [2]Dušan Vapa, [1]Miljen Maletin and [3]Isidora Radosavkić

[1]Centre of Forensic Medicine, Toxicology and Molecular Genetics, Clinical Center of Vojvodina, Novi Sad 21000, Serbia
[2]Department of Forensic Medicine, Faculty of Medicine, University of Novi Sad, Novi Sad 21000, Serbia
[3]Centre of Laboratory Medicine, Clinical Centre of Vojvodina, Novi Sad 21000, Serbia

Abstract

Background and Objective: Ricin is a naturally occurring toxin and one of the most accessible and most lethal poisons. Castor oil plants are proved to be not only a source of ricin, but also a toxic alkaloid ricinine, which is considered to be a biomarker for the intoxication of castor oil plant or ricin itself. The purpose of this experiment was to determine the distribution and concentration of ricinine in the blood and tissues of experimental rats after their treatment with castor seeds suspension. **Materials and Methods:** Experimental rats were simultaneously orally tested with the castor seeds suspension which contained sub-lethal concentration of ricin and sacrificed after 6, 12, 24, 36 and 48 hrs. The gas chromatography-mass spectrometry method was developed to quantify ricinine in the samples of blood, brain, lungs, kidneys and liver. **Results:** The obtained experimental results were processed by the data augmentation method to achieve better data variability and make more reliable conclusions. The highest content of ricinine in blood was measured 6 hrs after the treatment, as well as in the lungs and kidneys. In these samples, the concentration of ricinine decreased over time. In the final stage of the experiment, the highest ricinine concentration was registered in brain tissue. **Conclusion:** The obtained experimental results offer the possibility of selecting the samples of blood and brain tissue to prove acute poisoning and the cause of death due to ricin intoxication.

Key words: Ricin poisoning, ricinine, gas chromatography-mass spectrometry, forensic toxicology, forensic medicine, biological weapon

Citation: Radosavkić, R., M.Đ. Brenesel, V. Pilija, D. Vapa, M. Maletin and I. Radosavkić, 2024. Ricinine distribution in blood and tissue after oral intoxication-The data augmentation approach. Int. J. Pharmacol., 20: 54-61.

Correspondi ng Author: Radosav Radosavkić, Centre of Forensic Medicine, Toxicology and Molecular Genetics, Clinical Center of Vojvodina, Novi Sad 21000, Serbia

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Ricin is considered as one of the most lethal natural poisons known[1-3]. According to some sources ricin is 6000 times more poisonous than cyanide and 12000 times more poisonous than rattlesnake venom[4]. It is obtained from the castor bean plant (*Ricinus communis*), which grows in a tropical climate and whose seeds contain its highest content (1-5%)[5,6]. Ingestion of castor seeds is surely the most common cause of ricin poisoning, although inhalation and injection proved to be the most potent routes[7]. Owing to protein destruction in the alimentary tract, toxicity of ricin is lower after the oral intake[8]. Intact castor seeds can easily travel through digestive tract without any symptoms of poisoning. The degree of seed mastication is closely connected to the level of toxicity of the ingested seeds[9]. Ricin is observed as a potential weapon of bioterrorism since the plant *Ricinus communis* is easily available and the extraction of ricin from castor bean seeds is quite simple. Since it poses a significant risk for human health, it is classified as a biological weapon B[10,11]. It has also been recently used for the formation of immunotoxins in the therapy of malignant diseases and AIDS[12,13]. Castor oil plants are proved to be not only a source of ricin, but also a toxic alkaloid ricinine (0.3-0.8%) which makes them the only known natural source of ricinine[14]. Seeds of this plant are used for the co-extraction of ricinine and toxalbumin ricin as well[15]. Detection of ricinine in clinical samples is relatively simple by using different chromatographic methods. Nevertheless, its identification in biological samples is complicated, which makes ricinine a biomarker for the intoxication by castor oil plant or ricin itself[16]. Studies in experimental animals are always limited by number of animals included regarding ethics issues. This can increase uncertainties regarding the results obtained and conclusions made, especially in statistics. Thus, the data augmentation approach is an efficient mathematical way of obtaining large sets of results that simulate the variability of experimental results, as well as the variability of obtained conclusions[17].

This study aimed to examine the distribution of ricinine as a biomarker for ricin exposure in the blood and tissues of experimental animals at different times after their treatment with castor seeds suspension. Furthermore, the experimentally obtained results were additionally augmented by Monte Carlo simulations to minimize the uncertainties regarding the limited number of experimental animals used. The conducted study should facilitate the selection of tissues with the highest content of ricinine and by analysis of human biological samples, would contribute in proving acute poisoning and the cause of death due to ricin intoxication.

MATERIALS AND METHODS

Study area: The study was conducted from January to March, 2022 in the National Reference Laboratory for Rabies-Pasteur Institute in Novi Sad and in the Center for Forensic Medicine, Toxicology and Molecular Genetics, Clinical Center of Vojvodina, Novi Sad, Serbia.

Herbal material: Castor beans (200 beans) for treating experimental animals were collected in the garden of a private property in the area of Bačka, AP Vojvodina, Serbia. Quantitative Gas Chromatography-Mass Spectrometry (GC-MS) analysis showed that the content of ricinine in castor beans was 0.15%, which is to literature data[14]. Based on that, the content of ricin in the seeds was about 0.9% or about 1.60 mg. Given that the LD_{50} of ricin in laboratory rats is 30 mg kg^{-1}, it was decided to treat the animals with a suspension of three seeds of castor bean in 1 mL of physiological solution, which represents a dose of 19 mg kg^{-1} b.wt., of ricin, i.e. 3.2 mg kg^{-1} b.wt., of ricinine.

Experimental animal treatment: Experiments were carried out on white sexually 30 mature male Wistar rats body weight 250-350 g. The animals were selected by random choice method from the vivarium (Military Medical Academy, Belgrade, Serbia). Until the start of the experiment, experimental animals were kept in cages in groups of two or three, in a room with a 12 hrs light-dark cycle and an ambient temperature of 20-25 °C. They had free access to commercial pelletized food and water. For treating experimental animals dried castor beans were decorticated, washed with tap water and then ground in a mortar with 1 mL physiological solution (0.9% NaCl, Hemofarm, Serbia). A suspension of three seeds was prepared for each animal.

The animals were divided into two groups:

- **Control group:** The 5 animals upon weighing, received 1 mL of physiological solution and sacrificed after 48 hrs
- **Experimental group:** The 25 animals upon weighing received 1 mL of the prepared suspension of three seeds of castor bean (19 mg kg^{-1} b.wt., of ricin, i.e. 3.2 mg kg^{-1} b.wt., of ricinine) and sacrificed after the different times: 6, 12, 24, 36 and 48 hrs, each time 5 animals

Castor bean suspension was administered using a stainless-steel probe, opening size 18 g, for per-oral treatment of experimental animals and after that the animals were kept in separate cages. After a certain period, animals were anesthetized with a 25% solution of urethane (Sigma-Aldrich-Germany), in a dose of 5 mL kg^{-1} intraperitoneally. After the loss of the righting reflex, animals were sacrificed by exsanguination (cardiopuncture) and the following samples were taken: Blood (in tubes with anticoagulant lithium heparin, BD Vacutainer, Great Britain), brain, lungs, kidneys and liver. Samples were stored at -80°C until analysis.

Samples preparation: To blood samples (2 mL), a solution of the internal standard (IS) of meperidine (0.1 mL; 0.1 μg mL^{-1}) was added together with solid buffer K_2CO_3: $NaHCO_3$ = 2:3 and 25% NH_4OH to obtain pH = 9 and the content was diluted to 15 mL with distilled water.

Quartz sand (1 g) and sodium chloride (1.5 g) were added to 30 brain samples (each 1.5 g) and then homogenized in a mortar. After adding the solution of meperidine-IS (0.1 mL; 0.1 g mL^{-1}) to homogenates, they were diluted with 15 mL of acidified distilled water (pH 2, adjusted by adding 2 m H_2SO_4). To remove cholesterol and higher fatty acids, extraction was carried out with n-hexane (5 mL) on a Vortex T-Genie 2 apparatus for 2 min. Organic layers were discarded after centrifuging at 3000 rpm for 10 min, while aqueous layers were adjusted to pH = 9 by adding solid buffer K_2CO_3: $NaHCO_3$ = 2:3 and 25% NH_4OH.

Samples of lungs (2 g), kidneys (2 g) and liver (12 g) were homogenized in a mortar and after adding a solution of the IS of meperidine (0.1 mL; 0.1 μg mL^{-1}) were diluted with 15 mL of acidified distilled water (pH = 2, adjusted by adding 2M H_2SO_4) and heated on a hot plate at 80°C for 1 hr. After acid hydrolysis solid buffer K_2CO_3: $NaHCO_3$ = 2:3 and 25% NH_4OH were added to adjust pH = 9.

Diluted blood samples and brain, lungs, kidneys and liver homogenates were extracted by solid phase extraction (SPE) using Extrlut NT 20 extraction columns (Merck, Germany). Elution was done with the solvents methylenechloride/methanol = 9:1 and after evaporating to dryness and reconstitution in methylene chloride, samples were analysed by GC-MS method.

Development and validation of GC-MS analytical method: Analysis were performed using Agilent gas chromatograph (GC) with mass selective detector (MS) as described Djurendic-Brenesel *et al.*[18]. The MS was run in SCAN mode (m/z 50-550) for qualitative analysis and SIM mode (selected ion monitoring) for quantitative analysis (m/z 164, 121, 82 for ricinine and 247, 172, 218 for IS of meperidine).

Separate stock solutions of ricinine and IS (meperidine) were prepared at the concentration 100 μg mL^{-1} in distilled water by diluting the original standard solutions (in methanol) of ricinine (1 mg mL^{-1}) and meperidine (1 mg mL^{-1}), purchased from Sigma-Aldrich, Germany. Ricinine working solutions were prepared from stock solution in the concentration range of 0.02-0.50 μg mL^{-1}. The internal standard working solution was prepared at the final concentration of 0.1 μg mL^{-1}.

Calibration samples-spiked blood (2 mL), brain (1.5 g), lungs (2 g), kidneys (2 g) and liver (12 g) control samples were prepared by adding aliquots of standard working solutions of ricinine to final concentrations from 0.02 to 0.50 μg mL^{-1} and 0.1 mL of standard working solutions of IS. All calibration samples were extracted according to the described method above.

Quantification was carried out based on the characteristic m/z values of ions for ricinine. The ratio of the peak areas of ricinine and that of IS were presented as a function of the substance concentration using linear regression method, the coefficient of correlation being r^2 = 0.997-0.999. The lower detection limit for ricinine was 0.007 μg mL^{-1} and limit of quantitation was 0.02 μg mL^{-1}.

Recovery was determined by adding a known amount of ricinine to the control bio-logical samples, which were then treated on SPE columns and after the GC-MS analysis, the peak areas of ricinine were compared with peak areas of the same amount of the ricinine injected directly to the GC-MS instrument without extraction. This way recovery achieved the range of 80-90%.

Ethical consideration: In this study, all protocols and experiments were approved by the Institutional Animal Care and Use Committee (No. III-2011-07).

Statistical analysis: All of the obtained results were summarized by application of MS Office Excel (v. 2019) and further statistically processed by application of Tibco Statistica. The results were analyzed by application of descriptive statistics, whereas the linear correlation between the groups of interest was evaluated using Pearson's correlation coefficient. A value of $p<0.001$ was considered statistically significant. The effects of sampling time on the ricinine concentration in different biological samples were further examined by the application of multivariate statistics (principal component analysis). Principal Component Analysis (PCA) is a dimension reduction technique that enables a better understanding of the original dataset variability by presenting it in the low-dimensional space defined by principal components. However, the limited number of the obtained

analytical results for ricinine concentrations in the present study indicated the application of a simple data augmentation method. For this purpose, Monte Carlo simulations in Crystal Ball software (v 11.1; Oracle Corporation, Austin, TX) with 500 iterations were applied to analytically determined ricinine concentrations, whereas the assumption of Gaussian distribution was applied to data. This approach enabled boosting of the initial data matrix described by 25 cases and 5 variables to the dataset with dimensions 2500×5 which served as an input for PCA.

RESULTS

Ricinine quantification: Figure 1 shows the content (average value, n = 5) of experimentally determined ricinine in samples of blood, lungs, kidneys, liver and brain of male rats measured 6, 12, 24, 36 and 48 hrs after the treatment. The data are presented as mean values for 5 experimental animals in the group (n = 5).

In Fig. 1, the highest content of ricinine after 6 hrs of treatment was measured in samples of blood (4.25±0.78 ng mL^{-1}), brain (3.80±0.44 ng g^{-1}), lungs (3.75±0.27 ng g^{-1}) and kidneys (2.56±0.16 ng g^{-1}), while the lowest was registered in the liver (1.86±0.28 ng g^{-1}). Between 6 and 12 hrs, a decrease in ricinine concentration is observed in blood, lungs and kidneys, while concentration in the brain and liver increases. In the next 12 hrs, ricinine concentration rapidly decreases in the brain and slightly decreases in the blood and other tissues. The 24 hrs after the intoxication, ricinine concentrations in blood, lungs, kidneys and brain are lower than at the beginning of the experiment, which was contrary to concentration observed in the liver. It is important to mention that the concentration in the brain (3.60±0.23 ng g^{-1}) has a much higher value compared to other tissues (1.37-2.52±0.14 ng g^{-1}) and blood (1.82±0.15 ng mL^{-1}). Between 24 and 36 hrs, ricinine level slightly increases in the liver and kidneys, while it decreases in the blood and other tissues. The concentration of ricinine is the highest in the liver 36 hrs after the intoxication and has the same value as in the brain. In the final stage of the experiment (48 hrs after the treatment), the highest ricinine concentration was registered in the brain (2.05±0.14 ng g^{-1}), while it was the lowest in the lungs (0.98±0.13 ng g^{-1}). At the same time, the content of ricinine in blood, liver and kidneys is approximately on the same level.

A statistically significant positive correlation was found for the values of concentration of ricinine in the blood and lungs ($p<0.001$), blood and kidneys ($p<0.001$) and lungs and kidneys ($p<0.001$). No statistically significant correlation was established between values of concentration of ricinine in liver and other tissues, as well as in the brain and other tissues (Table 1).

Data augmentation-chemometric approach: The application of principal components analysis on a dataset describing ricinine distribution in different biological samples during time after treatment shows that the first two principal components

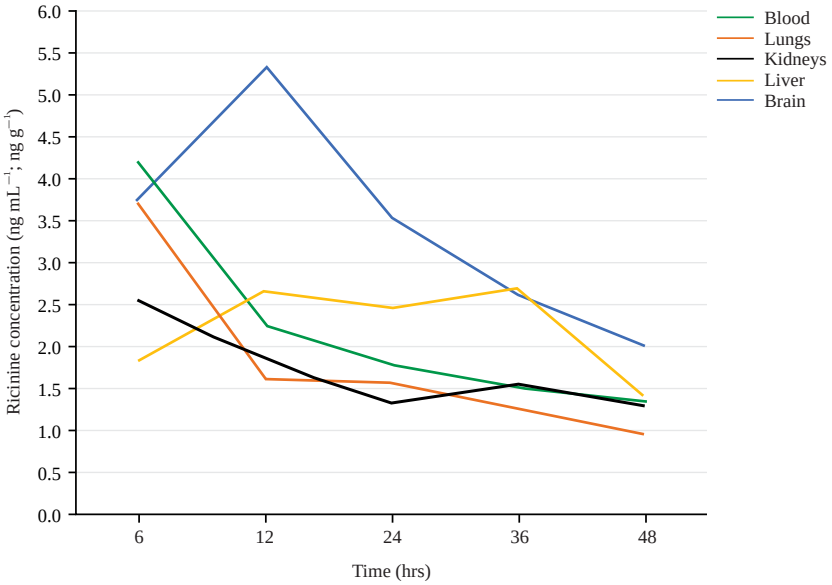

Fig. 1: Concentration of ricinine (average value, n = 5) in samples of blood and tissues vs time

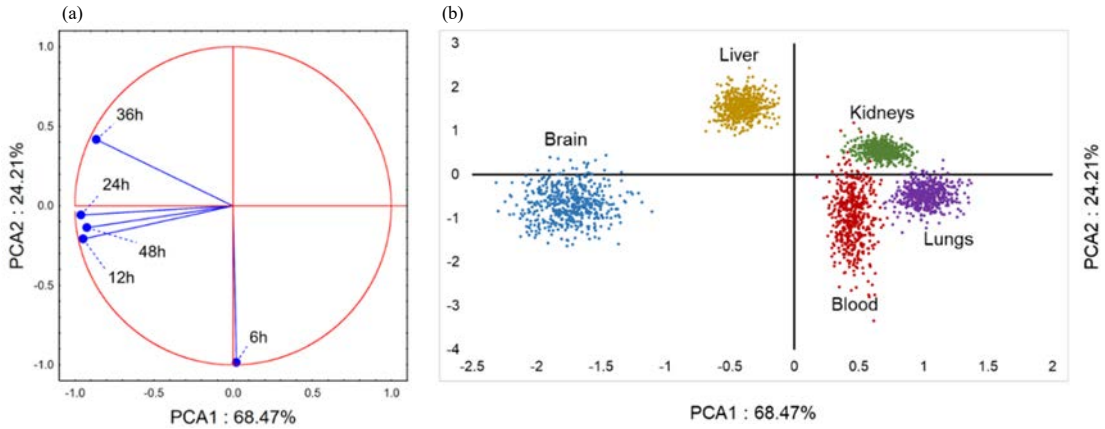

Fig. 2(a-b): PCA of ricinine concentrations, (a) PCA loadings and (b) the position of analyzed cases in the space define by the first two principal components

Table 1: Correlation of ricinine concentration between different tissues

		Blood	Lungs	Kidneys	Liver	Brain
Blood	r	1	0.936	0.869	-0.171	0.347
	p		<0.001	<0.001	0.436	0.104
Lungs	r		1	0.877	-0.215	0.277
	p			<0.001	0.324	0.200
Kidneys	r			1	-0.137	0.382
	p				0.532	0.072
Liver	r				1	0.505
	p					0.014
Brain	r					1
	p					

(PCA1 and PCA2) describe more than 90% of the original dataset variability (Fig. 2a). The size of the variability in term of PCA1 is mostly described by ricinine concentrations determined in evaluated biological samples at later stages of intoxication (12, 24 and 48 hrs). On the other hand, the shape of obtained results variability in terms of PCA2 mostly correlates to the ricinine concentrations determined at the first time point (6 hrs) after treatment. The position of the evaluated samples in the space defined by the first two principal components (Fig. 2a) indicates a close grouping of kidney, blood and lung samples in the positive part of PCA1. This is a direct consequence of rapid decrease of recorded ricinine concentrations in the periods after treatment, as well as ricinine lower level when compared to two other two types of biological samples. Furthermore, if the aforementioned group was observed, a separative grouping of kidney samples in term of PCA2 can be noticed as a consequence of lower ricinine levels recorded 6h after intoxication. On the other hand, liver and brain samples are located in the negative part of PCA1 as a consequence of higher recorded ricinine concentrations during the observed time frame. Of particular importance is the highest level of ricinine in brain samples which is present 12, 36 and 48 hrs after intoxication. On the other hand, the position of liver samples in the positive part of PCA2 indicates lower ricinine levels 6 hrs after intoxication, but grouping in the same space with brain samples suggests higher ricinine concentration in time points 12, 24 and 36 hrs, when compared to blood, kidney and lungs samples (Fig. 2b).

DISCUSSION

This work aimed to examine the distribution of ricinine as a reliable marker of ricin poisoning in the blood and inner organs of experimental animals. To this end the procedures of preparation and application of castor seed suspension to laboratory rats were developed, as well as of isolation of ricinine from the biological samples of blood and inner organs (lungs, kidneys, liver and brain) and its GC-MS quantification. The animals were orally treated with the castor seed suspension containing sub-lethal concentration of ricin, which represents a novelty concerning previous studies in which animals were treated with pure substances, ricin or ricinine.

So far, a large number of methods for proving ricin in biological samples have been described. One of the earliest techniques was radioimmunoassay (RIA), which could be used to detect low concentrations of ricin[19]. Enzyme-Linked Immunosorbent Assay (ELISA) involves a shorter assay time and advantages in specificity and simplicity[20]. However, assay time is greatly improved by the use of various techniques such as fluorescence-based fiber optics or colloidal gold particles[21,22]. Capillary electrophoresis coupled with mass

spectrometry allows distinction between different forms of ricin toxin and also allows rapid separation and purification of complex mixtures[23]. In the case of mass exposure to a biotoxin weapon the microfluid chip-based immunoassay could be a very useful method because it works rapidly, requires minimal sample volume and shows noticeable sensitivity[24]. The IPCR (immune-polymerase chain reaction) is a very sensitive antigen detection method that is used for the detection of ricin in human serum samples and with the use of IPCR the limit of detection of an ELISA was increased up to 10000-fold[25]. Liquid Chromatography-Electrospray/Mass Spectrometry (LC-ES/MS) and Matrix-Assisted Laser Desorption/Ionization Time-of-Flight (MALDI-TOF) method are in use for the detection of ricin and ricinine from crude plant material[26]. Newer methods of ricin detection are based on immunocapture by anti-B-chain antibodies and determination of the adenine released by the A chain[27].

Detection of ricin in biological samples can be done indirectly through the detection of ricinine. Ricinine is a small size molecule that can be detected in clinical samples using various chromatographic methods[28].

Following the resorption through the digestive tract, ricin and ricinine enter the blood and reach the highest concentrations within the first hours after the intoxication. Earlier studies showed that ricin can be found in blood within less than one hour after oral intake[29,30]. Results of current study experiments show that the concentration of ricinine is the highest in blood 6 hrs after a suspension is applied and then sharply decreases between 6 and 12 hrs. That was following literature data which showed that soon after the intoxication there is a sharp decrease of the ricin level in blood and that is most probably the consequence of its rapid binding to receptors on the cell surface and redistribution along tissues[31-34].

Variations in the ricinine level in the lungs have similar dynamics as in the blood. Interestingly, concentrations of ricinine in the lungs and brain are almost identical 6 hrs after the intoxication. That can be explained by the fact that ricinine passes through blood-brain and respiratory barrier and quickly redistributes to the brain and lungs from blood. It is necessary to point out that the toxicokinetic data of ricinine distribution after oral ingestion are rare. The numerous studies are based on intravenous or inhalation route of toxin application. Results of the study by Hodges *et al.*[35] and Sapoznikov *et al.*[36] showed that after ricin aerosol application to mice its concentration in lungs decreased rapidly within the first 6 hrs, followed by a constant slight decrease until 30 hrs. Similar results were obtained by Ramsden *et al.*[32] and Godal *et al.*[33], who applied ricin to animals intravenously or intraperitoneally. Ishiguro *et al.*[37] applied ricin to laboratory rats orally in a dose of 10 mg kg^{-1} and they established that it cannot be detected in the lungs between 6 and 72 hrs after intoxication.

Current study results show that the distribution of ricinine in kidneys is by previous studies[38,39]. There is a case report about the suicidal attempt of a man who took a castor seeds suspension orally and intravenously. The concentration of ricinine in his urine had a similar progression as in our research. In that case, the highest concentration in urine was detected 12 hrs after the intoxication, when the first sample for the analyses was taken (in our experiment it was 6 hrs after the intoxication). Similarly, the concentration of ricinine decreased rapidly in the period of up to 29 hrs after the intoxication, while in the subsequent period of 29 to 41 hrs, it increased slightly[40].

In the current study, while the concentration of ricinine decreases in blood, it increases in liver, showing the redistribution pathway. Unlike other analyzed tissues, the concentration of ricinine remains constant in the liver for a certain period. This can be explained by the accumulation of toxin in the liver which most likely happens through portal bloodstream and its subsequent metabolism. Results of the present study experiment are by the study by Ishiguro *et al.*[41], who also established that the concentration of ricin increases in the liver starting from 6 hrs after the oral intoxication, reaching its maximum value after 48 hrs and then decreasing. In case reports of Verougstraete *et al.*[31] and Staňková *et al.*[42] ricinine is detected in the liver in post-mortem samples after a suicidal intravenous injection of castor bean extract.

There is no literature data about ricin or ricinine distribution in brain tissue after intoxication. Results of current study show high levels of ricinine in the brain in all analyzed periods, with the highest concentration 12 hrs after intoxication. Ricinine remains in a high concentration in brain tissue several hours after intoxication, compared to other tissues.

Cases of ricine intoxication are rare in our practice. Symptoms of ricine poisoning are non-specific, so reliable data about castor beans ingestion are very important for establishing the diagnosis of poisoning.

CONCLUSION

This study indicates that in cases with the suspicion of ricin poisoning it is necessary to take biological samples for chemical-toxicological analyses, especially blood and urine within 12 hrs after the intoxication. The obtained results also show that ricinine can be identified in blood and urine samples 48 hrs after the intoxication, but the concentrations

in that period are significantly lower. If the ricin poisoning is fatal, it is necessary to confirm that the cause of death is a consequence of ricin intoxication. Results of our research show that during the autopsy it is necessary to take samples of blood, urine, lungs, kidneys, liver and brain because ricinine, as an indirect marker of ricin intoxication, can be found in these organs. The death caused by ricin poisoning most often happens in the period of 36 to 72 hrs after intoxication and based on our results it can be concluded that ricinine can be detected with a high level of certainty first of all in liver and brain tissue, because the highest ricinine concentrations were registered in these organs. According to our knowledge, this is the first study in which ricinine distribution is described after oral intoxication with castor bean suspension. The obtained experimental results are of importance as they offer the possibility of selecting liver and brain tissues as representative samples which analysis would contribute to resolving the causes of death due to ricin intoxication.

SIGNIFICANCE STATEMENT

The main goal of this research was to prove the presence of ricinine in the blood of laboratory rats with the time interval that passed from the oral application of the suspension, as well as to determine the distribution and concentration of ricinine in the organs of laboratory rats at different times of sacrifice. The results of the research enable the standardization of procedures for selecting representative samples in case of suspicion of ricin poisoning and methods of proving acute poisoning.

REFERENCES

1. Delgado, M.L.O., A. Avril, J. Prigent, J. Dano and A. Rouaix *et al*., 2021. Ricin antibodies' neutralizing capacity against different ricin isoforms and cultivars. Toxins, Vol. 13. 10.3390/toxins13020100.
2. Polito, L., M. Bortolotti, M.G. Battelli, G. Calafato and A. Bolognesi, 2019. Ricin: An ancient story for a timeless plant toxin. Toxins, Vol. 11. 10.3390/toxins11060324.
3. Hoyt, K., J.R. Barr and S.R. Kalb, 2021. Detection of ricin activity and structure by using novel galactose-terminated magnetic bead extraction coupled with mass spectrometric detection. Anal. Biochem., Vol. 631. 10.1016/j.ab.2021.114364.
4. Lim, H., H.J. Kim and Y.S. Cho, 2009. A case of ricin poisoning following ingestion of Korean castor bean. Emerg. Med. J., 26: 420-767.
5. Bradberry, S.M., K.J. Dickers, P. Rice, G.D. Griffiths and J.A. Vale, 2003. Ricin poisoning. Toxicol. Rev., 22: 65-70.
6. Franke, H., R. Scholl and A. Aigner, 2019. Ricin and *Ricinus communis* in pharmacology and toxicology-from ancient use and "Papyrus Ebers" to modern perspectives and "poisonous plant of the year 2018". Naunyn-Schmiedeberg's Arch. Pharmacol., 392: 1181-1208.
7. Audi, J., M. Belson, M. Patel, J. Schier and J. Osterloh, 2005. Ricin poisoning: A comprehensive review. JAMA, 294: 2342-2351.
8. Benamor, M., E. Gharbi, S. Bouzid, O. Chakroun-Walha and N. Rekik, 2020. Ricin poisoning after oral ingestion of castor beans: A case report and literature review. Afr. J. Emerg. Med., 10: 274-276.
9. Hamelin, E.I., R.C. Johnson, J.D. Osterloh, D.J. Howard and J.D. Thomas, 2012. Evaluation of ricinine, a ricin biomarker, from a non-lethal castor bean ingestion. J. Anal. Toxicol., 36: 660-662.
10. Worbs, S., B. Kampa, M. Skiba, E.M. Hansbauer and D. Stern *et al*., 2021. Differentiation, quantification and identification of abrin and *Abrus precatorius* agglutinin. Toxins, Vol. 13. 10.3390/toxins13040284.
11. Janik, E., M. Ceremuga, J. Saluk-Bijak and M. Bijak, 2019. Biological toxins as the potential tools for bioterrorism. Int. J. Mol. Sci., Vol. 20. 10.3390/ijms20051181.
12. Sandvig, K., S. Kavaliauskiene and T. Skotland, 2021. The protein toxins ricin and shiga toxin as tools to explore cellular mechanisms of internalization and intracellular transport. Toxins, Vol. 13. 10.3390/toxins13060377.
13. Wen, J., M. Yan, Y. Liu, J. Li and Y. Xie *et al*., 2016. Specific elimination of latently HIV-1 infected cells using HIV-1 protease-sensitive toxin nanocapsules. PLoS ONE, Vol. 11. 10.1371/journal.pone.0151572.
14. Johnson, R.C., S.W. Lemire, A.R. Woolfitt, M. Ospina, K.P. Preston, C.T. Olson and J.R. Barr, 2005. Quantification of ricinine in rat and human urine: A biomarker for ricin exposure. J. Anal. Toxicol., 29: 149-155.
15. Abbes, M., M. Montana, C. Curti and P. Vanelle, 2021. Ricin poisoning: A review on contamination source, diagnosis, treatment, prevention and reporting of ricin poisoning. Toxicon, 195: 86-92.
16. Isenberg, S.L., M.D. Carter, M.A. Miller, A.I. Noras and M.A. Mojica *et al*., 2018. Quantification of ricinine and abrine in human plasma by HPLC-MS-MS: Biomarkers of exposure to ricin and abrin. J. Anal. Toxicol., 42: 630-636.
17. MacGowan, A.P., 2004. Elements of design: The knowledge on which we build. Clin. Microbiol. Infect., 10: 6-11.
18. Djurendic-Brenesel, M., N. Mimica-Dukic, V. Pilija and M. Tasic, 2010. Gender-related differences in the pharmacokinetics of opiates. Forensic Sci. Int., 194: 28-33.
19. Ramakrishnan, S., M.R. Eagle and L.L. Houston, 1982. Radioimmunoassay of ricin A- and B-chains applied to samples of ricin A-chain prepared by chromatofocusing and by DEAE Bio-Gel A. Biochim. Biophys. Acta Gen. Subj., 719: 341-348.

20. Dayan-Kenigsberg, J., A. Bertocchi and E.A.E. Garber, 2008. Rapid detection of ricin in cosmetics and elimination of artifacts associated with wheat lectin. J. Immunol. Methods, 336: 251-254.
21. Narang, U., G.P. Anderson, F.S. Ligler and J. Burans, 1997. Fiber optic-based biosensor for ricin. Biosens. Bioelectron., 12: 937-945.
22. Shyu, R.H., H.F. Shyu, H.W. Liu and S.S. Tang, 2002. Colloidal gold-based immunochromatographic assay for detection of ricin. Toxicon, 40: 255-258.
23. Na, D.H., C.K. Cho, Y.S. Youn, Y. Choi, K.R. Lee, S.D. Yoo and K.C. Lee, 2004. Capillary electrophoresis to characterize ricin and its subunits with matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. Toxicon, 43: 329-335.
24. Meagher, R.J., A.V. Hatch, R.F. Renzi and A.K. Singh, 2008. An integrated microfluidic platform for sensitive and rapid detection of biological toxins. Lab Chip, 8: 2046-2053.
25. Lubelli, C., A. Chatgilialoglu, A. Bolognesi, P. Strocchi, M. Colombatti and F. Stirpe, 2006. Detection of ricin and other ribosome-inactivating proteins by an immuno-polymerase chain reaction assay. Anal. Biochem., 355: 102-109.
26. Ferranti, P., C. Nitride and M. Gallo, 2017. Omic Analysis of Protein and Peptide Toxins in Food. In: Analysis of Food Toxins and Toxicants, Wong, Y.C. and R.J. Lewis (Eds.), John Wiley and Sons Limited, Hoboken, New Jersey, ISBN: 9781118992685, pp: 1-36.
27. Liang, L.H., X. Cheng, H.L. Yu, Y. Yang and X.H. Mu *et al*., 2021. Quantitative detection of ricin in beverages using trypsin/Glu-C tandem digestion coupled with ultra-high-pressure liquid chromatography-tandem mass spectrometry. Anal. Bioanal. Chem., 413: 585-597.
28. Thiebot, P., N. Maaziz, C. Oppon, L. Bertolo and R. Magny *et al*., 2023. Human plasma ricinine quantification by LC-HRMS after micro-solid-phase elution. Biomed. Chromatogr., Vol. 37. 10.1002/bmc.5604.
29. Feldberg, L., E. Elhanany, O. Laskar and O. Schuster, 2021. Rapid, sensitive and reliable ricin identification in serum samples using LC-MS/MS. Toxins, Vol. 13. 10.3390/toxins13020079.
30. Cook, D.L., J. David and G.D. Griffiths, 2006. Retrospective identification of ricin in animal tissues following administration by pulmonary and oral routes. Toxicology, 223: 61-70.
31. Verougstraete, N., D. Helsloot, C. Deprez, O. Heylen, I. Casier and K. Croes, 2019. Lethal injection of a castor bean extract: Ricinine quantification as a marker for ricin exposure using a validated LC-MS/MS method. J. Anal. Toxicol., 43: e1-e5.
32. Ramsden, C.S., M.T. Drayson and E.B. Bell, 1989. The toxicity, distribution and excretion of ricin holotoxin in rats. Toxicology, 55: 161-171.
33. Godal, A., Ø. Fodstad, K. Ingebrigtsen and A. Pihl, 1984. Pharmacological studies of ricin in mice and humans. Cancer Chemother. Pharmacol., 13: 157-163.
34. Lefever, S., I. Geerts, E. Vermeulen, P. Vermeersch and F. de Rydt *et al*., 2021. Non-lethal intoxication by ingestion of 50 castor beans: serial measurement of ricinine in blood, plasma and urine. J. Anal. Toxicol., 45: e8-e12.
35. Hodges, A.L., C.G. Kempen, W.D. McCaig, C.A. Parker, N.J. Mantis and T.J. LaRocca, 2019. TNF family cytokines induce distinct cell death modalities in the A549 human lung epithelial cell line when administered in combination with ricin toxin. Toxins, Vol. 11. 10.3390/toxins11080450.
36. Sapoznikov, A., Y. Gal, R. Falach, I. Sagi and S. Ehrlich *et al*., 2019. Early disruption of the alveolar-capillary barrier in a ricin-induced ARDS mouse model: Neutrophil-dependent and-independent impairment of junction proteins. Am. J. Physiol. Lung Cell. Mol. Physiol., 316: L255-L268.
37. Ishiguro, M., M. Mitarai, H. Harada, I. Sekine, I. Nishimori and M. Kikutani, 1983. Biochemical studies on oral toxicity of ricin. I. Ricin administered orally can impair sugar absorption by rat small intestine. Chem. Pharm. Bull., 31: 3222-3227.
38. Bucaretchi, F., C.F. Borrasca-Fernandes, C.C. Prado, R. Lanaro and J.L. Costa *et al*., 2021. Near-fatal poisoning after ricin injection. Clin. Toxicol., 59: 158-168.
39. Bambauer, T.P., L. Wagmann, A.A. Weber and M.R. Meyer, 2021. Further development of a liquid chromatography-high-resolution mass spectrometry/mass spectrometry-based strategy for analyzing eight biomarkers in human urine indicating toxic mushroom or *Ricinus communis* ingestions. Drug Test. Anal., 13: 1603-1613.
40. Roen, B.T., A.M. Opstad, A. Haavind and J. Tonsager, 2013. Serial ricinine levels in serum and urine after ricin intoxication. J. Anal. Toxicol., 37: 313-317.
41. Ishiguro, M., S. Tanabe, Y. Matori and R. Sakakibara, 1992. Biochemical studies on oral toxicity of Ricin. IV. A fate of orally administered ricin in rats. J. Pharmacobio-Dyn., 15: 147-156.
42. Staňková, M., P. Handlos, M. Švidrnoch and V. Maier, 2020. Fatal intoxication by intravenous injection of castor bean (*Ricinus communis* L.) extract-A case study. Int. J. Legal Med., 134: 2133-2141.