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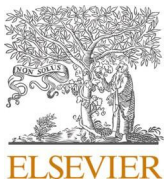

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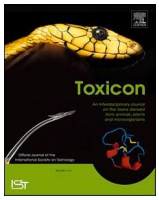

Gaps in forensic toxicological analysis: The veiled abrin

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ABSTRACT

Abrus precatorius is an herbaceous, flowering plant that is widely distributed in tropical and subtropical regions. Its toxic component, known as abrin, is classified as one of the potentially significant biological warfare agents and bioterrorism tools due to its high toxicity. Abrin poisoning can be utilized to cause accidents, suicides, and homicides, which necessitates attention from clinicians and forensic scientists. Although a few studies have recently identified the toxicological and pharmacological mechanisms of abrin, the exact mechanism remains unclear. Furthermore, the clinical symptoms and pathological changes induced by abrin poisoning have not been fully characterized, and there is a lack of standardized methods for jkjl; 'sdfkidentifying biological samples of the toxin. Therefore, there is an urgent need for further toxicopathologic studies and the development of detection methods for abrin in the field of forensic medicine. This review provides an overview of the clinical symptoms, pathological changes, metabolic changes, toxicologic mechanisms, and detection methods of abrin poisoning from the perspective of forensic toxicology. Additionally, the evidence on abrin in the field of forensic toxicology and forensic pathology is discussed. Overall, this review serves as a reference for understanding the toxicological mechanism of abrin, highlighting the clinical applications of the toxin, and aiding in the diagnosis and forensic identification of toxin poisoning.

1. Introduction

Abrus precatorius, a plant widely distributed in tropical and subtropical regions, is primarily found in China, specifically in the Hainan, Guangxi, Yunnan, and Taiwan provinces (Fig. 1). The seeds of A. precatorius plants possess a hard texture, a stunning gloss, and a long-lasting red color, making them suitable for creating decorative items. All parts of A. precatorius, including the seeds, roots, stems, and leaves, are highly toxic, with the seeds being the most toxic. The toxicity is primarily due to a toxin called abrin, which constitutes approximately 2.8%–3.0% of all components in the seeds (Qian et al., 2022). The lethal dose 50 of abrin in mice is 0.04 μg/kg, while in human adults, it ranges from 0.1 to 1.0 μg/kg (Dickers et al., 2003). Notably, abrin is reported to be > 70 times more toxic than ricin and is classified as one of the potentially most significant biological warfare agents and bioterrorism tools (Tiwari et al., 2017).

Abrin is a type II ribosome-inactivating protein, with a molecular weight of 60–65 kDa. The toxin consists of two polypeptide chains (A and B chains) which are connected by a disulfide bond (Fig. 2) (Cheng et al., 2010; Peng et al., 2022). The A chain (RNA-N-glycosidase) has a molecular weight of 30 kDa and contains 251 amino acids divided into three folded structural domains. It is catalytically active. On the other hand, the B chain has a molecular weight of 35 kDa and contains 267 amino acids. It is a galactose-specific lectin that facilitates the binding of abrin to cell membranes. As a result, abrin can irreversibly inactivate ribosomes through site-specific depurination, leading to cell death (Olsnes, 2004). There are four isoforms of abrin: abrin-a, abrin-b, abrin-c, and abrin-d. Although these forms are encoded by different genes, they belong to the same polygenic family, all containing A- and B-chain structures. Abrin-b and abrin-c exhibit weak cytotoxic effects due to their low B-chain agglutination activity. However, abrin-a and abrin-d are highly cytotoxic (Janik et al., 2019).

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A. precatorius has a long history of medicinal use in China, as documented in traditional Chinese medicine records such as the *National Compendium of Chinese Herbal Medicine*, *Dictionary of Traditional Chinese Medicine*, and *Chinese Materia Medica*. These records indicate that *A. precatorius* is effective in clearing heat, removing toxins, and acting as a diuretic. The roots and vines of the plant are specifically used for treating sore throat and hepatitis, while its leaves are used for bronchitis (Li et al., 2014). Additionally, African folk medicine records mention the use of *A. precatorius* for various conditions such as diarrhea, skin infections, sexually transmitted infections, dysentery, and gonorrhea (Taur et al., 2011). Furthermore, studies have shown that abrin, a compound found in A. precatorius, possesses antitumor, antihistamine, antiallergic, and antimicrobial properties (Chopra et al., 2019; Garaniya and Bapodra, 2014; Hirschberger et al., 2017; Singh and Singh, 1999; Zhang, 2022).

The widespread use of *A. precatorius* is associated with numerous reports of medical accidents, misuse, suicide, and abrin-related poisoning (Table 1). However, only a few poisoning cases have been reported in detail, and the mechanism by which abrin induces toxicity remains unclear. Furthermore, there is currently no consensus on the testing standards for detecting this toxin. These limitations present significant challenges for forensic practitioners when dealing with poisoning cases related to abrin (Dickers et al., 2003; Olsnes, 2004). Here, we summarized recent literature on abrin to provide an overview of the manifestations of *A. precatorius* poisoning, the underlying toxicological mechanisms, and the available detection methods. Additionally, we explored the research of abrin in forensic toxicology and forensic pathology. Collectively, this review can provide an overview of the toxic mechanisms of *A. precatorius* and serve as a reference for its clinical use, poisoning diagnosis, and forensic identification.

2. The symptoms of abrin poisoning

Abrin poisoning is primarily caused by ingesting its seeds. Currently, there are no effective antidotes available for treating abrin poisoning in clinical settings, so supportive therapy is the main approach for alleviating abrin poisoning (Huang et al., 2017; Kumar et al., 2023). The severity of toxic effects of abrin largely depends on the condition of the seeds when ingested. Ingesting intact seeds usually does not lead to clinical toxicity because the hard outer shell prevents the digestion and absorption of abrin. However, if the shell is damaged, the released components can induce severe toxicity in humans (Ninan and James, 2019). Additionally, developing seeds have a softer shell compared to mature seeds, making ingestion of such developing seeds more likely to cause toxicity (Hart, 1963).

2.1. Clinical symptoms

After ingestion, abrin typically has an incubation period ranging from 6 h to 3 days, depending on the route and dose (Fernando, 2001; Jang et al., 2010; Rinner et al., 2021; Sarkar et al., 2017). Poisoning by abrin is primarily characterized by hemorrhagic gastroenteritis with erosions. Early symptoms commonly include nausea, vomiting, watery diarrhea, abdominal cramps, loss of appetite, melena, toxic hepatitis, acute renal failure, hemolysis, cyanosis, and retinal hemorrhage leading to visual impairment (Alhamdani et al., 2015; Patil et al., 2016). Additionally, neurologic symptoms such as headache, hallucinations, dilated pupils, hand and foot twitching, lethargy, coma, seizures, and encephalitis-like syndromes may also occur (Rajaratnam et al., 2022). Some individuals may also exhibit atypical symptoms, such as demyelinating encephalitis, cerebral edema, increased intracranial pressure, basal ganglia hemorrhage, and optic nerve papillae edema (Sahni et al., 2007; Sahoo et al., 2008; Subrahmanyan et al., 2008). In a case report by Sahni et al., a 30-year-old female intentionally ingested 3–4 crushed

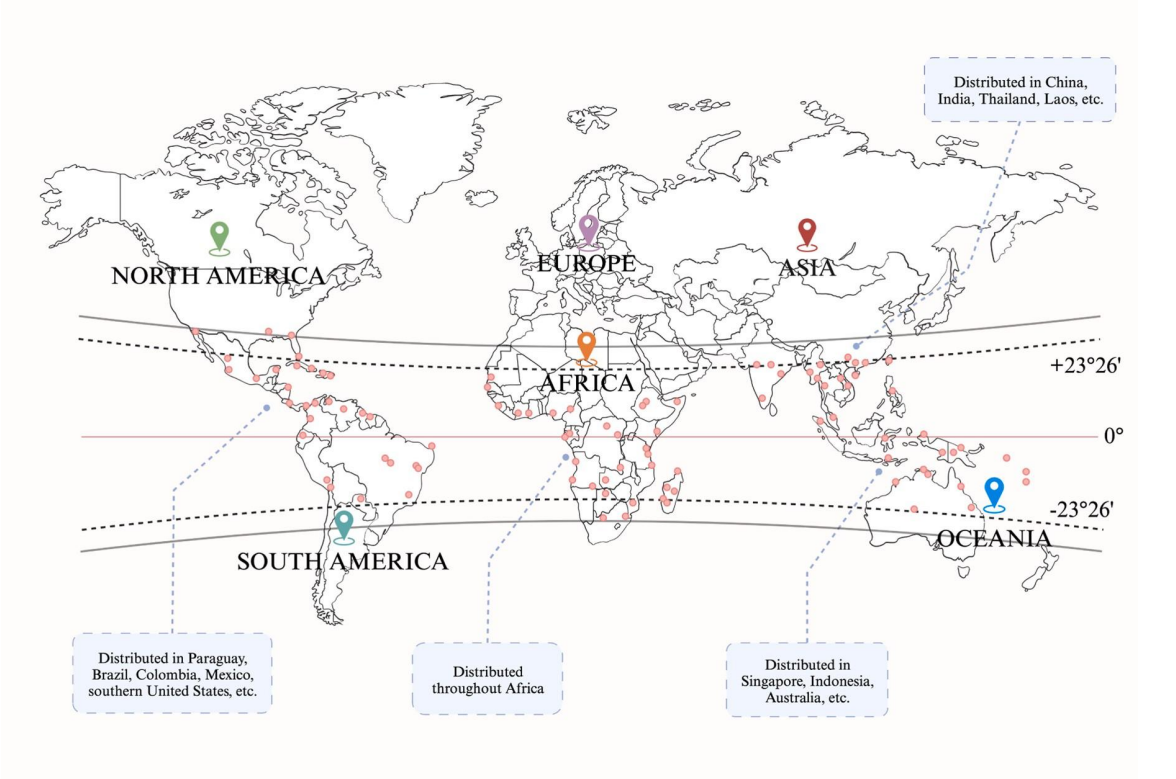

Fig. 1. Global distribution of *A. precatorius*.

seeds of *A. precatorius*, which resulted in coma, paralysis of the third motor nerve, and increased intracranial pressure (Sahni et al., 2007). Additionally, magnetic resonance imaging scans of the brain showed signs of demyelination. Consequently, the patient was diagnosed with acute demyelinating encephalitis and passed away 3 days later (Sahni et al., 2007). Due to the similarity of symptoms between abrin poisoning and ricin poisoning, it is essential to conduct a combination of laboratory tests and examination of clinical manifestations in order to establish an accurate diagnosis.

2.2. Pathological changes

The pathological changes associated with abrin poisoning are not easily noticeable. Previous animal experiments and post-mortem examinations have shown that cases of abrin poisoning exhibit edema and hemorrhage in the gastrointestinal tract, as well as congestion and edema in the parenchymal tissues (Fig. 3) (Chaturvedi et al., 2015; Phatak et al., 2019; Rinner et al., 2021). Additionally, signs of edema and intravascular leukocytosis have been observed in the brain tissue. The heart tissue has shown intravascular leukocytosis and elevated troponin levels, but no necrosis of cardiomyocytes. The toxin has also induced intravascular leukocytosis and fibrin thrombi in the lung tissues, without interstitial or alveolar pulmonary edema. Liver toxicity is characterized by leukocytosis in the blood sinuses, steatosis, and ischemic necrosis in the centers of lobules. Moreover, the gastrointestinal tract exhibits submucosal congestion and plasma membrane petechial hemorrhage, without mucosal lesions or intraluminal hemorrhages. In cases of abrin poisoning, the spleen shows congestion. The kidneys of patients with *A. precatorius* poisoning present glomerular capillary fibrin thrombi and acute tubular epithelial necrosis. The toxin also affects the adrenal glands by mainly inducing cortical hemorrhage and necrosis (Chaturvedi et al., 2015; Phatak et al., 2019; Rinner et al., 2021).

Rinner et al. (2021) reported a case study involving a 35-year-old male who attempted suicide by injecting a filtrate made from the aqueous slurry of 150 *A. precatorius* seeds subcutaneously and intramuscularly. The patient was admitted to the hospital 17.5 h after the injection and unfortunately passed away after 4 days of receiving supportive care. Autopsy findings indicated hemorrhage at the injection site, muscle necrosis, and hemorrhage in the right axillary lymph node, along with vascular fibrin-like necrosis and thrombosis. However, the most critical histopathological changes observed in this patient included skeletal muscle necrosis and severe interstitial hemorrhage at the right forearm (injection site), hemorrhage and lymphovascular necrosis in the right axillary lymph node, significant intravascular leukocytosis in the lungs, fibrin thrombosis of the glomerular capillaries, hemorrhage and necrosis of the adrenal cortex, and hepatocellular steatosis and necrosis. Polarized light examination did not reveal any accumulation of foreign material at the injection site or in other tissues. The autopsy and histopathological findings mentioned above bear resemblance to the well-known Georgi Markov case of ricin poisoning. While ricin and abrin have similar toxicological profiles, ricin is found to be less toxic (Crompton and Gall, 1980). Hence, it is crucial to emphasize the distinction between *A. precatorius* poisoning and ricin poisoning when identifying such cases.

2.3. Metabolic changes

Abrin can enter the human body through oral ingestion, inhalation, or injection (Griffiths et al., 1995). Animal studies have demonstrated that ingestion of abrin leads to significant changes in various hematological, biochemical, inflammatory, and oxidative stress-related parameters (Makdasi et al., 2019; Sant et al., 2017; Sant et al., 2019; Sapoznikov A et al., 2022). These changes primarily include an increase in leukocyte, neutrophil, and platelet counts, as well as a decrease in lymphocyte count and hematocrit percentage. The toxin also significantly reduces hemoglobin levels and erythrocyte count. In terms of biochemical parameters, abrin ingestion leads to a significant increase in serum ammonia, alkaline phosphatase (ALP), bilirubin, total bilirubin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), urea, uric acid, and creatinine levels. Conversely, it significantly decreases levels of glutathione, albumin, total protein, glucose, and cholesterol. Additionally, the toxin causes a significant increase in levels of tumor necrosis factor (TNF-α) and interferon, a decrease in total antioxidant status, an increase in lipid peroxidation (LPO) and malondialdehyde (MDA, the end-product of LPO) levels, and an increase in the

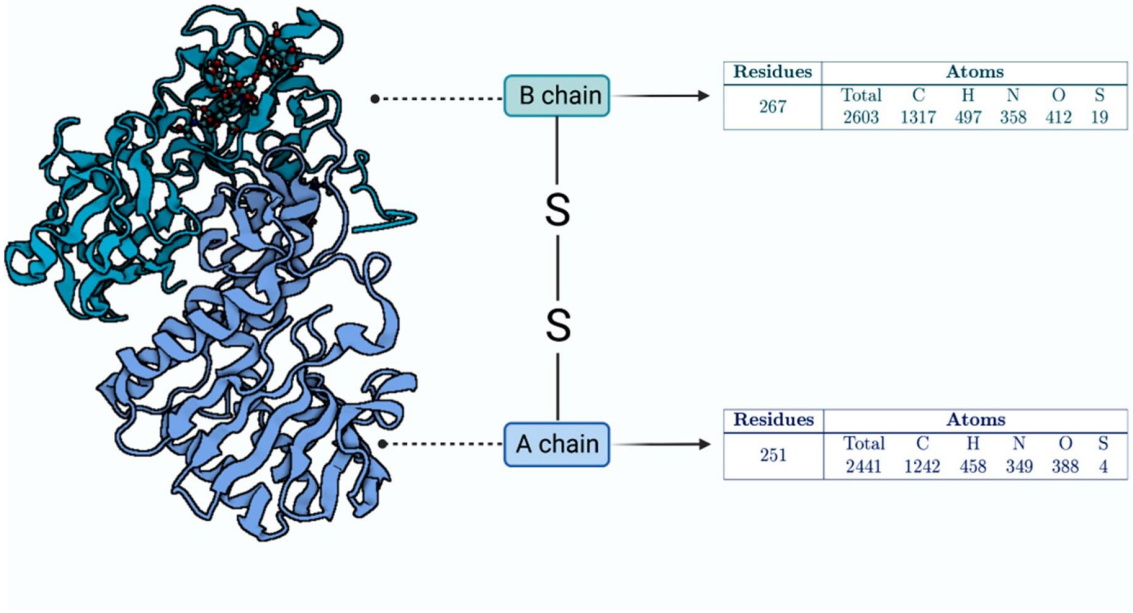

Fig. 2. Molecular structure of abrin.

Table 1
Summary of case reports of abrin poisoning and the clinical manifestation.

Country	Year	Avenues	Case Information	intake (quantity)	Poisoning manifestations	Time of death	Recovery time	References
Sri Lanka	2001	profess conviction	13 years male, Accidental ingestion	two or more jequirity beans	Nausea, vomiting, diarrhea, abdominal cramps, vomiting blood, melena, pulmonary edema, sinus tachycardia, and hypertension	–	9 days	Fernando (2001)
India	2005	profess conviction	42 years male, Ingested deliberately for aphrodisiac purposes	some seeds	Bloody diarrhea, abdominal pain, fever, coma with depressive reflexes and plantar subsidence, constriction of both sides of the pupil, and seizures	–	2 weeks	Pillay et al. (2005)
India	2007	profess conviction	30 years female, Intentional ingestion for abortion	three to four seeds	Oppressive pain of the abdominal wall, vomiting, loose stools, coma, seizures, dilated pupil of the right eye, and demyelinating encephalitis	102 h	–	Sahni et al. (2007)
India	2008	profess conviction	19 years male, Ingested on purpose because of a quarrel with his father	a handful (10–15) of crushed Abrus precatorius seeds	Abdominal pain, vomiting, watery diarrhea with blood, epigastric pain and tenderness, seizures, drowsiness, and decreased speech	–	15 days	Sahoo et al. (2008)
India	2008	profess conviction	17 years female, Intentional ingestion due to family arguments	ten crushed seeds	Vomiting, loose watery stools with blood, epigastric pain and pressure, drowsiness, decreased speech, renal failure, hyponatremia, and hypokalemia	–	1 month	Subrahmanyan et al. (2008)
India	2008	profess conviction	28 years female, Intentional ingestion	seven crushed seeds	Vomiting, diarrhea, generalized tonic–clonic seizures, and dyspnea	4 days	–	Subrahmanyan et al. (2008)
India	2009–2015	profess conviction	112 patients(>13 years),	crushed seeds (58.40%)	Vomiting, loose stools, blood in stools, abdominal pain, fever, altered sensation, seizures, and tremors	six patients expired (5.35%)		Karthikeyan and Amalnath (2017)
America	2010	profess conviction	20 years male, suicide	10 seeds	Nausea, vomiting, watery diarrhea, and hypokalemia	–	3 days	Jang et al. (2010)
America	2015	profess conviction	18 months boy, Accidental ingestion	Unknown number of seeds	Vomiting and diffuse abdominal tenderness	–	4 days	Alhamdani et al. (2015)
India	2016	profess conviction	2 years boy, Accidental ingestion	Unknown number of seeds	Reflex and plantar flexor depression, bilateral optic nerve papilla edema, generalized tonic–clonic seizures, upper gastrointestinal hemorrhage, and acute renal failure	11 days	–	Patil et al. (2016)
China	2017	profess conviction	16 years female, Intentional ingestion	10 crushed seeds	Nausea, vomiting, abdominal pain, diarrhea, black liquid stools, bloody urine with decreased urine output, dizziness, and fatigue	–	5 days	Huang et al. (2017)
India	2017	profess conviction	2 years boy, Accidental ingestion	Unknown number of seeds	Convulsions, lethargy, hypertonia of limbs, moderately dilated pupils bilaterally, and unresponsiveness	–	2 weeks	Sarkar et al. (2017)
India	2019	profess conviction	20 years male, suicide	Powdered seeds	Nausea, vomiting, diarrhea, vomiting blood, blood in stool, tachycardia, confusion, hallucinations, asymmetry of gaze, abnormal rotation of the eyeballs, medial deviation of the right eye, and slight tremor of the upper extremities	5 days	–	Horowitz et al. (2020)
India	2019	profess conviction	22 years female, suicide	Unknown number of seeds	Abdominal pain, vomiting, diarrhea, fever, generalized tonic–clonic seizures, and tachycardia		27 days	Ninan and James (2019)
India	2020	injection	35 years male, suicide	an aqueous slurry of 150 A. precatorius seeds	Erythema, edema, fever, and pressure pain at all injection sites, sclerosis of the forearm, decreased mental status, autonomic dysfunction, tachycardia, and seizures	84 h	–	Rinner et al. (2021)
Sri Lanka	2022	profess conviction	17 years female, Intentional ingestion as a result of a domestic dispute	four seeds	Lower abdominal cramps, watery diarrhea, generalized tonic–clonic seizures, flaccid extremities, and cognitive dysfunction	–	1 month	Rajaratnam et al. (2022)
The Netherlands	2022	profess conviction	58 years male, suicide	Unknown number of seeds	–	–	4 days	(Dibbits et al., 2022)
India	2023	profess conviction	43 years female, suicide	25 crushed seeds	Abdominal pain, vomiting, watery stools, and tachycardia	–	8 days	Kumar et al. (2023)

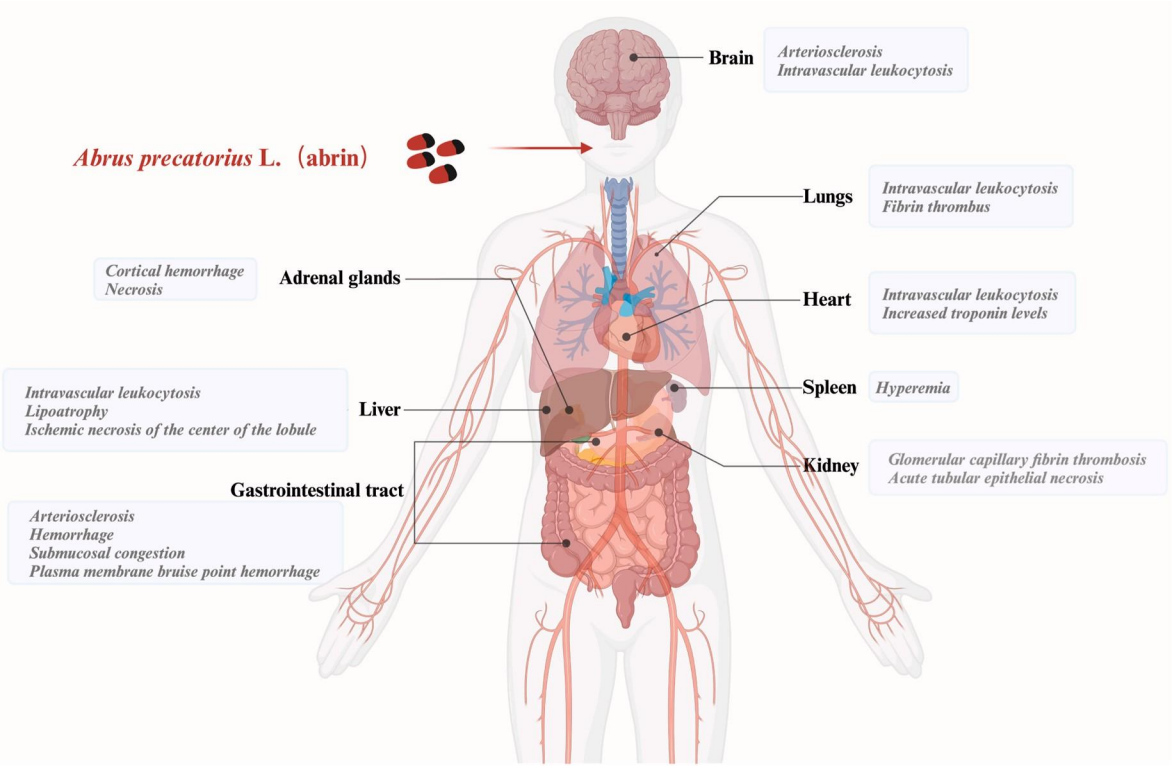

Fig. 3. Pathological changes caused by abrin poisoning.

level of thiobarbituric acid (TBA) active substance produced by MDA and TBA (Makdasi et al., 2019; Sant et al., 2017; Sant et al., 2019; Sapoznikov A et al., 2022).

Another characteristic sign of abrin poisoning is the capillary leakage syndrome (Dickers et al., 2003). The toxin induces an increase in leukocytes and a decrease in blood albumin, which coincides with the development of a systemic inflammatory response syndrome. This leads to increased vascular permeability and plasma leakage, with albumin entering tissues and causing hypoalbuminemia. Additionally, abrin poisoning elevates bilirubin levels, which may be associated with hepatic dysfunction and the breakdown of hemoglobin. The toxin also leads to significant increases in serum AST, ALT, and ALP levels, primarily due to the release of these enzymes from damaged hepatocytes into the bloodstream, indicating severe liver toxicity. Renal impairment is another effect of the toxin, as seen by significant increases in urea and uric acid levels. The decrease in glucose and cholesterol levels may serve as indicators of toxin-induced liver damage (Huang et al., 2017; Sant et al., 2017).

The changes in metabolic indicators indicate that abrin has detrimental effects on various organs in the human body. Therefore, it is crucial for forensic toxicology research to detect the distribution of abrin in the body. According to previous reports, abrin is quickly distributed to all tissues and organs in humans through blood circulation. The liver contains the highest amount of abrin, followed by the blood, lungs, spleen, kidneys, and heart (Dickers et al., 2003).

2.4. Unique characteristics of abrin poisoning

While the symptoms of abrin poisoning can be similar to other types of poisoning, there are some unique characteristics (including the pathological features) that can facilitate the professional diagnosis of the condition. The best-known distinguishing features of abrin poisoning include the following clinical symptoms and biochemical manifestations. 1. Early gastrointestinal symptoms: People who have been poisoned by abrin will typically experience gastrointestinal symptoms such as vomiting, diarrhea, and abdominal pain within a few hours of ingestion. These symptoms can be severe and may be accompanied by dehydration and electrolyte imbalances. 2. Delayed hepatic and renal failure: In severe cases of abrin poisoning, liver and kidney damage can occur. This can lead to jaundice, ascites (fluid buildup in the abdomen), and other complications. 3. Lack of fever: Unlike many other types of poisoning, abrin poisoning does not typically cause a fever. 4. Biochemical manifestation: There are some specific laboratory findings that can help to diagnose abrin poisoning. These include an elevated white blood cell count, elevated liver enzymes, and elevated creatinine levels (Alhamdani et al., 2015; Dickers et al., 2003; Karthikeyan and Amalnath, 2017; Patil et al., 2016; Rajaratnam et al., 2022). Nonetheless, there is a current lack of understanding of unique pathological features associated with abrin poisoning, which is a major obstacle in the forensic diagnosis.

3. Toxicological mechanisms of action

Abrin is a member of type II ribosome-inactivating proteins, and it produce toxic effect through irreversible inactivation of protein synthesis via a polynucleotide adenosine glycosylase mechanism (Bolognesi et al., 2016; Tiwari and Karande, 2019). Structurally, abrin consists of a heterodimer composed of two polypeptides: the catalytically active A chain and a B chain with galactose-specific lectin properties. The A chain is responsible for the toxicity of abrin as it induces depurination of adenine at position A4324 in the 28S ribosomal RNA through RNA-N-glycosidase activity. This depurination inhibits protein synthesis and ultimately leads to cell death (Melchior and Tolleson, 2010). Additionally, the B chain binds the toxin to cell surface glycoproteins or glycolipids, facilitating the endocytosis of the toxin (Deeks et al., 2002). Once inside the cell, a portion of abrin is transported retrogradely to the endoplasmic reticulum, where the disulfide bond between the A and B chains is cleaved. Subsequently, the A chain enters the cytoplasm and binds to the cytotoxic gene loop (α-sarcin loop) of 28S ribosomal RNA, thereby inhibiting protein synthesis (Gadadhar et al., 2014). There is

also evidence suggesting that abrin induces apoptosis through disrupting different cellular pathways, including the mitochondrial, endoplasmic reticulum, death receptor, and lysosomal pathways (Shih et al., 2001; Wu et al., 2010).

3.1. Mitochondrial pathway

Narayanan et al. were the first to report that abrin induces apoptosis through the mitochondrial pathway in Jurkat cell line derived from a human T-cell leukemia (Narayanan et al., 2004). Their study demonstrated that the toxin stimulates the binding of BH3-only proteins and inhibits anti-apoptotic proteins such as BCL-2, BCL-XL, and MCL1. Additionally, abrin activates pro-apoptotic proteins BAX and BAK, leading to a decrease in mitochondrial membrane potential, enhanced permeability, and release of cytochrome C (Cyt C) into the cytoplasm. This interaction with apoptotic protease activating factor-1 forms the apoptotic complex, recruiting and activating pro-caspase-9 to form caspase-9. Subsequently, caspase-9 activates effector caspase-3 and caspase-7, initiating a caspase-dependent cascade reaction that induces apoptosis (Liu et al., 2012). According to Papo et al., abrin causes overproduction of reactive oxygen species, resulting in the opening of the mitochondrial permeability transition pore and a decrease in mitochondrial membrane potential (Papo and Shai, 2005). This leads to the release of Cyt C from the inner mitochondrial membrane into the cytoplasm, ultimately leading to cascade activation and apoptosis. Furthermore, Momoi et al. discovered that abrin triggers the cleavage of BH3-interacting structural domain of the pro-apoptotic protein BH3 by activating caspase-2 and caspase-8 (Momoi, 2004). This subsequently induces the loss of mitochondrial membrane potential and the activation of downstream effectors (caspase-9 and caspase-3), linking the death receptor pathway to the mitochondrial pathway (Fig. 4A).

3.2. Death receptor pathway

The study by Saxena et al. identified the Fas/Fas-L pathway as an important mediator of abrin-induced apoptosis (Saxena et al., 2013). Fas-associated death domain (FADD) is a key mediator of the Fas pathway, regulating cellular sensitivity to Fas-mediated apoptosis by modulating effector caspases. Saxena et al. demonstrated that abrin affects FADD-dependent death receptor pathway by stimulating the binding of FAS to FAS-L and recruiting pro-caspase-8 and pro-caspase-10 for activation. Activated caspase-8 and -10 in turn trigger the activation caspase-3 and caspase-7 to execute apoptosis. Additionally, abrin induces the cleavage of BID into tBID, which inhibits anti-apoptotic proteins (BCL-2, BCL-X_L, and MCL1) and activates pro-apoptotic proteins (BAX and BAK). This ultimately enhances mitochondrial membrane permeability, activates the mitochondrial pathway, and induces apoptosis (Fig. 4A). The expression of FADD increases following exposure to the toxin, further confirming the involvement of the Fas pathway in abrin-induced apoptosis (Saxena et al., 2013).

3.3. Endoplasmic reticulum pathway

In the context of cell signaling, the endoplasmic reticulum serves as a crucial player in apoptosis. In mammalian cells, the endoplasmic reticulum (ER) houses key components of the unfolded protein response (UPR), namely inositol-requiring enzyme 1 (IRE1), transmembrane protein kinase-like endoplasmic reticulum kinase (PERK), and activating transcription factor 6 (ATF6) (Hebert and Molinari, 2007; Schro¨der and Kaufman, 2005). These transmembrane sensors and proteins are responsible for protein homeostasis by upregulating molecular chaperones. However, persistent endoplasmic reticulum stress (ERS) triggers apoptotic pathways, leading to cellular damages with defective protein folding.

According to a previous study, the phaseolus toxin A chain inhibits protein synthesis, causing unfolded proteins to accumulate in the ER (Mishra and Karande, 2014). The accumulation of unfolded proteins triggers UPR and ERS. Immunoglobulin heavy-chain binding protein in pre-B cells (BIP) and 78-kD glucose-regulated protein (GRP78) dissociate from UPR sensors (PERK, IRE1, and ATF6), and polymerize with the accumulated unfolded proteins. This further activates ATF6, sheared X-box binding protein 1-spliced (XBP1s), and activating transcription factor 4 (ATF4) to trigger apoptosis. ATF6, an endoplasmic reticulum type II transmembrane protein kinase, translocates from the ER to the Golgi apparatus upon dissociation from BIP/GRP78. In the Golgi apparatus, ATF6 is hydrolyzed by site-1 protease and site-2 protease cleavage. Activated ATF6 stimulates the expression of C/EBP homologous protein (CHOP) to induce apoptosis. IRE1, an endoplasmic reticulum type I transmembrane protein kinase, forms a homodimer with ribonucleic acid endonuclease activity upon dissociation from BIP/GRP78. This homodimer specifically cleaves XBP1 mRNA precursor to form the active transcriptional activator XBP1s. XBP1s upregulates CHOP protein expression and induces apoptosis. On the other hand, PERK undergoes dimerization and autophosphorylation, leading to the phosphorylation of eukaryotic initiation factor 2 (eIF2α) at serine 51. Phosphorylated eIF2α activates the synthesis of ATF4 mRNA, which promotes the expression of CHOP genes upon entry into the nucleus. CHOP then activates growth arrest and DNA damage-inducible 34, decreases the expression of BCL-2, and results in BAX translocation from the cytoplasm to the mitochondria, ultimately inducing the mitochondrial apoptotic pathway (Fig. 4B) (Mishra and Karande, 2014).

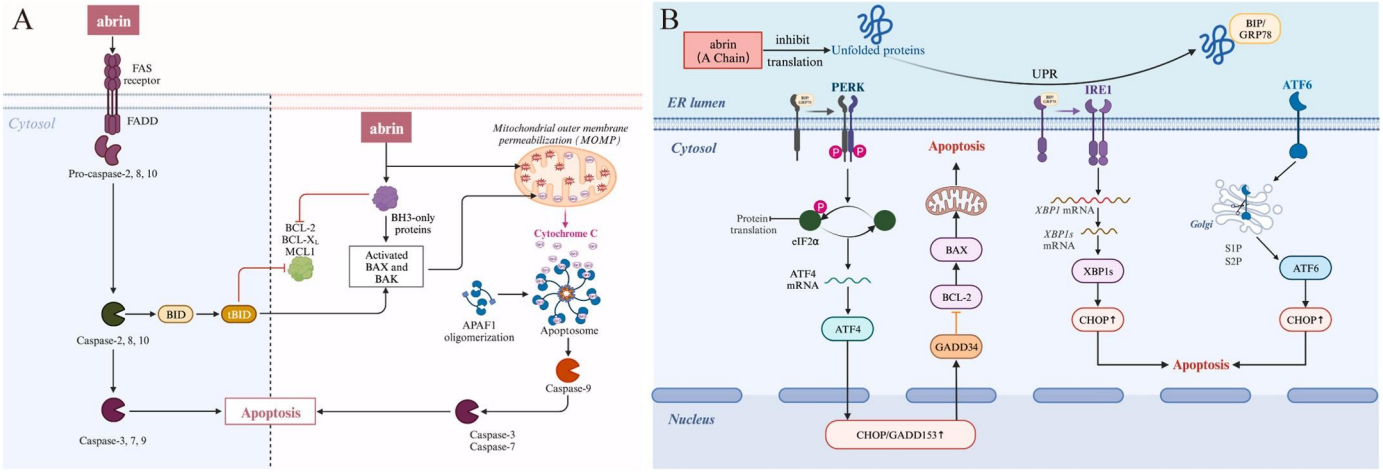

Fig. 4. Mechanisms underlying the toxicity of abrin. (A) Mitochondrial pathway and death receptor pathway; (B) endoplasmic reticulum pathway.

3.4. Lysosomal pathway

Several studies indicate that abrin can induce necrosis by increasing the permeability of lysosomal membranes and releasing the proteases. Additionally, the toxin triggers apoptosis in a caspase-independent manner. Bora et al. conducted a study to investigate various signaling processes, including the loss of mitochondrial membrane potential, the production of reactive oxygen species, and the alteration of lysosomal membrane permeability, in order to understand the mode of action by abrin. Intriguingly, the results suggested that lysosomes and histones are involved in *A. precatorius*-induced cell death (Bora et al., 2010). When the integrity of the lysosomal membrane is disrupted, proteases are released from lysosomes to degrade different intracellular substances. Thus, lysosomes may be implicated in caspase-independent cell death caused by abrin (Tang et al., 2008). The most abundant lysosomal proteases are cysteine protease, histone B, histone L, and aspartic protease histone D, with histone B and D playing a significant role in abrin-induced cell death (Guicciardi et al., 2004).

3.5. Other pathways

A study conducted by Wenhe Zhu et al. aimed to investigate the mechanism by which abrin induces damage to human lung epithelial cells. The researchers utilized transcriptomics, proteomics, and metabolomics data to understand the mechanism of action. Several signaling pathways including TNF, Rap1, apoptosis, MAPK, nod-like receptor, and Ras seem involved in the damages caused by abrin. Activation of these pathways by abrin are associated with various effects, such as inflammation, oxidative stress, metabolic dysregulation, and changes in cellular function. This comprehensive analysis shed light on the underlying mechanism of action of the toxin (Zhu et al., 2019).

4. Detection methods

4.1. Mass spectrometry

Abrin and L-abrine are present in the plant seeds in absolute amounts of 0.12% and 0.45%, respectively (Qian et al., 2022). L-abrine, which has not been found in other plants, is considered a specific biomarker for *A. precatorius* (Laskar et al., 2019). Currently, L-abrine is commonly used in laboratories to determine the presence of *A. precatorius* or poisoning from its toxin. The specific assay involves the extraction of L-abrine using solid-phase extraction techniques, followed by quantitative analysis using high-performance liquid chromatography–mass spectrometry (Dodge et al., 2015; Johnson et al., 2009; Owens and Koester, 2008).

Wooten et al. showed that L-brine can be reliably and sensitively detected in urine within the first 24 h after exposure to the toxin (Wooten et al., 2014). In a clinical case study, Rinner et al. found that L-abrine was detected in urine 24.5 h after injection of a filtrate made from the aqueous seriflux of *A. precatorius* seeds. However, L-abrine was not detected in both urine and blood samples at 57.5 h post-injection (Rinner et al., 2021). In a case report by Horowitz et al., L-abrine was detected in urine but not in serum samples after 87 h of oral administration of large quantities of mature *A. precatorius* seeds (Horowitz et al., 2020). However, the detection time of L-abrine in the body varies significantly depending on the administration route of abrin (Isenberg et al., 2018). Therefore, it is important to fully understand the route of abrin entry, the time of entry, and the sample type to be utilized in order to select the optimal test protocol for L-abrine detection.

4.2. Real-time quantitative polymerase chain reaction (qPCR) assay

The production of high-purity abrin requires sophisticated instrumentation and specialized knowledge. Therefore, in real-life cases, offenders are more likely to use crude extracts containing plant-specific nucleic acids. These nucleic acids of abrin can be detected using qPCR (Bhaskar et al., 2012).

Felder et al. developed a method using OmniMix HS bead PCR reagents for detecting DNA molecules of abrin (Felder et al., 2012). They employed a novel primer and hybridization probe and utilized the 5′-nuclease technique on a SmartCycler instrument. The researchers determined that the detection limit for abrin DNA molecules was 1.2 genome copy numbers, with a sensitivity optimized through thorough analytical optimization. Additionally, the method was validated by detecting abrin DNA molecules in food samples. Although antibody-based immunoassays are currently the standard technique for abrin detection, qPCR offers a rapid and reliable alternative by detecting DNA molecules in samples (Bhaskar et al., 2012; Wang et al., 2004).

4.3. Immunoassays

4.3.1. Enzyme-linked immunosorbent assay (ELISA)

Abrin can be identified through its active chemical structure or related compounds found in *A. precatorius* seeds, such as L-abrine (Duracova et al., 2018; Johnson et al., 2009; Ramage et al., 2014). Worbs et al. (2021) have developed highly specific monoclonal antibodies against abrin, which have been used to establish two sandwich ELISAs for its detection. These ELISAs have a limit of detection of 22 pg/mL. This detection method is cost-effective, automatable, suitable for high-throughput analysis, and effective for testing complex samples like beverages and foods (Garber et al., 2008; He et al., 2017; Xu et al., 2015; Zhou et al., 2012). However, it does have a drawback of being time-consuming, with detection times ranging from 4 to 6 h when using microtiter plates (Gao et al., 2012; Xu et al., 2015; Yang et al., 2011).

4.3.2. Microfluidic chip-based immunodetection

Bai et al. utilized a microelectromechanical system to fabricate a unique nano-forested silicon microstructure, which was employed in the development of a novel microfluidic sensor chip. This sensor chip was equipped with a capillary self-driving function and a large area (Bai et al., 2022). Subsequently, the researchers combined the sensor chip with a double antibody sandwich immunoassay to establish an effective method for detecting abrin. Notably, the sensitivity of the sensor chip towards abrin was significantly higher compared to conventional immunochromatographic strips, with a limit of detection of≦10 pg/mL. Furthermore, the method exhibited high specificity and demonstrated good linearity in the range of 10–6250 pg/mL, even when faced with interferences from fruit juice or milk. The enhanced nano-forested silicon microstructure and the homogeneous color signal of the sensor chip contributed to a rapid detection time of less than 15 min. Consequently, this technique proves valuable for the swift detection of abrin in the biosafety field.

4.3.3. Up-converting phosphor technology-based lateral-flow assay (UPT-LFA)

Liu et al. developed a UPT-LFA-based rapid assay for the detection of abrin in food (Liu et al., 2016). The assay demonstrated high specificity for abrin, with a sensitivity of 0.1 ng/mL for standard toxin solutions. It also exhibited good linearity ($r = 0.9983$) for quantification in the concentration range of 0.1–1000 ng/mL. Moreover, the method showed excellent sample tolerance, with low limits of detection at 0.5–10 ng/g for solid and powdered samples and 0.30–0.43 ng/mL for liquid samples when analyzing various food samples spiked with *A. precatorius*. This method enables non-specialists to complete the entire process (from sample handling to result reporting) within 20 min. Thus, UPT-LFA is considered to be a rapid, sensitive, and reliable on-site method for determining abrin levels in food.

4.4. Chromogenic enzyme sensor

Cho et al. developed a portable colorimetric assay for the rapid detection of abrin (Cho and Jaworski, 2014). The method involved using

an immobilization strategy of unnatural amino acid site-specific coupling technology to create a magnetic bead system based on N-methyltryptophan oxidase. This system can detect the biomarker L-abrine in urine at concentrations as low as 4 μM of abrin. Additionally, the assay is highly portable and provides visual results. Therefore, this assay offers significant advantages for rapid assessment and toxicology management, especially in situations where specialized toxicology laboratories are not available.

4.5. Fluorescence quenching effect

Fluorescence quenching effect is a recently developed approach for abrin detection. This method is based on the interaction between the target molecule and fluorescently labeled aptamers on magnetic microspheres. The minimum detection limit for abrin using this method has been reported to be 5 ng/mL. Liu et al. utilized a four-nucleotide structure to characterize the abrin aptamer's arrangement of nucleotides. They subsequently created a quantitative structure-activity relationship model to determine the structure and affinity of the toxin aptamer. According to this model, the presence of abrin in the test material can be identified by screening toxin aptamers with high affinity through targeted mutagenesis (Liu et al., 2022). This method effectively overcomes the influence of complex interfering factors and can accurately detect abrin in water, soil, and food samples.

4.6. Other methods

Currently, there are several methods available for *in vivo* detection of toxins, such as ELISA, immunochromatographic strips, multiplexed immunoassays, electrochemiluminescence, and qPCR (Dodge et al., 2015; Garber et al., 2008; Pauly et al., 2012; Wang et al., 2004; Worbs et al., 2021; Yang et al., 2011). However, these methods have two lim- itations. Firstly, they are unable to determine whether the detected toxin is biologically active or inactivated/degraded. Secondly, the detection process requires expensive laboratory equipment, resulting in high detection costs. Thus, there is an urgent need for a low-cost assay that can detect the bioactivity of abrin and quantify it. In the study conducted by Rasooly et al., they addressed these issues to some extent. They utilized a low-cost CCD fluorescence sensor to measure cellular fluorescence, colorimetric, or luminescence intensity, with a minimum detection limit of 10 pg/mL (Rasooly et al., 2020). This method showed a 200-fold increase in sensitivity compared to the recently developed quantitative high-resolution targeted mass spectrometry technology (Rasooly et al., 2020). Chromatographic techniques, such as thin-layer chromatography (TLC), high-performance TLC, gas chromatography-mass spectrometry, and nuclear magnetic resonance, are commonly employed by forensic scientists to isolate and identify toxins from plant and animal samples. These techniques have also been adopted to analyze the active ingredients and chemical structures of abrin. Various components, including saponins, steroids, flavonoids, glycosides, and phenolic compounds, have been identified from abrin using these techniques (Hansbauer et al., 2017; Oladimeji et al., 2016; Pavithra et al., 2020; Singh et al., 2015; Verma et al., 2011). Additionally, infrared spectroscopic techniques can be used to screen toxic phytochemicals, although only a limited number of studies have implemented this method (Gowtham et al., 2019; Ji et al., 2019; Sharma S et al., 2023). For these techniques, standard references are crucial to provide landmarks in the spectrum for the accurate annotation of chemical components derived from abrin.

5. Conclusion and prospects

Due to the widespread presence of *A. precatorius* in tropical and subtropical regions and the high toxicity of abrin, cases of poisoning involving this toxin are of great concern to clinicians and forensic scientists. While several studies have examined the immunological aspects of abrin, there is a lack of research in the field of forensic toxicology. Abrin has the ability to induce apoptosis by inhibiting protein synthesis, although the exact mechanism remains unclear. Moreover, recent studies using systems biology techniques, such as proteomics and metabolomics, have made significant progress in deciphering the underlying mechanism of abrin-induced organ damages. These findings will serve as valuable guidance for further comprehensive investigations into the toxicological mechanism of this toxin.

The pathological changes associated with abrin poisoning have been assessed in limited number of studies. However, the specific pathological changes caused by this toxin are still enigmatic. In order to pinpoint the differences from ricin poisoning, future efforts are needed to delineate the holistic picture of clinical manifestations and pathologic changes caused by abrin poisoning. The understanding of the toxicological mechanisms of abrin may shed also light on the specific pathological alterations associated with this toxin. Currently, special attention should be paid in handling clinical cases to avoid the confusion of abrin poisoning and ricin poisoning. In cases of suspected abrin poisoning, the detection of biological samples such as blood and urine plays a crucial role in clinical medicine and forensic work. While there are several methods available to detect abrin, most of them are applicable only to toxins present in food and beverage samples. There is a need to develop reliable and sensitive methods for detecting abrin in biological samples in the future work.

Authorship contribution statement

Yinyu Chen: Conceptualization, Resources, Writing - original draft, Writing - review & editing. Jiaqi Liu: Conceptualization, Investigation, Writing - review & editing. Tao Song: Desk research, reviewing. Xing Zou: Desk research, reviewing. Qianyun Nie: Writing - review & editing, Supervision. Peng Zhang: Writing - review & editing, Supervision. All authors have read and agreed to the published version of the manuscript.

Ethics approval

This article dose not include any content for which ethical approval would need to be obtained.

Consent to participate

This article does not include any content for which informed consent would need to be obtained.

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CRediT authorship contribution statement

Yinyu Chen: Writing – original draft, Data curation, Conceptualization. **Jiaqi Liu:** Writing – review & editing, Investigation, Formal analysis, Data curation, Conceptualization. **Tao Song:** Project administration, Investigation, Formal analysis, Data curation. **Xing Zou:** Methodology, Funding acquisition, Formal analysis, Data curation. **Leilei Li:** Resources, Investigation, Data curation. **Qianyun Nie:** Writing – review & editing, Methodology, Formal analysis, Data curation. **Peng Zhang:** Writing – review & editing, Investigation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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