

Review Paper

Toxicology of *Dysosma versipallis* rhizome: A review

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According to the World Health Organization and the National Institutes of Health, Traditional Chinese medicine originated more than 3000 years ago. While there are quite obvious medical values suggesting that *Dysosma versipallis* rhizome is both desirable and achievable, there are still obstacles. Since the therapeutic dose and toxic dose are very close, *D. versipallis* rhizome poisoning cases were frequently reported. In this paper, the plant source, chemical constituent, toxicity reason, toxic dose, mechanism of toxicity, poisoning symptoms, pathological changes of body tissues after ingestion of *D. versipallis* rhizome poisoning and medical management are reviewed.

Key words: *Dysosma versipallis* rhizome, podophyllotoxin, toxicology, review.

INTRODUCTION

The '*Dysosma versipallis* rhizome', named *Rhizoma diphylleiae*, *Diphylleia sinensis* Li, was first recorded in 'Shennong's Herbal Classic' after the Podophyllum. Berberidaceae *Dysosma* plants, including *D. versipellis* (Hance) M. Cheng ex Ying, *D. pleiantha* (Hance) Woods, *D. veitchii* (Hemsl. et Wils.) Fu ex Ying, the rhizomes of which are used as Chinese herbal medicine. It mainly grows in regions of the Zhejiang province, Jiangxi province and Hubei province (Guo, 1992). And it is frequently used individually by external application to treat fracture, rheumatoid arthritis, lumbago, skelalgia, but it is not suitable for oral use (Zhang et al., 2007). Cases of poisoning, even death caused by misuse of *dysosma versipallis* rhizome were often reported in the past few years (Feng et al., 2003; Jin and Yuan, 2003; Fang and Hong, 2003; Chen, 2004; Li and Jiang, 2007; Gong et al., 2007). Since the chemical constituents of *Dysosma* plants are complicated, and the major constituent, Podophyllotoxin (Figure 1), is cytotoxic (Lv et al., 2006), it could prove extremely dangerous in case of overdose (Xiao, 2002). In order to guide the public in such poisoning cases, this article studied the toxicology

of *D. versipallis* rhizome by a thorough literature review.

TOXIC DOSE AND TOXIC CAUSE

Podophyllotoxin is the major poisonous constituent of *D. versipallis* rhizome, and its LD₅₀, analyzed by intragastrical administration to mice, is 90 mg/kg; by subcutaneous injection it is 24.6 mg/kg; by intraperitoneal injection it is 30 - 35 mg/kg (Xia, 2005). The LD₅₀ of powder of Yunnan *Dysosma* Rhizome (Podophyllum aurantiocaula Hand.-Mazz.) after intragastrical administration to mice is 0.493 g/kg, and a rabbit died about 12 h after intragastrical administration of the powders at a dose of 4.5 g/kg. Ethanol and water extract of tubers of *D. versipallis* rhizome at a dose of 1000 mg/kg (equivalently to tubers at dose of 20 g/kg) was injected intraperitoneally into mice, which lead to less active animal models and even death.

As the toxicity of *D. versipallis* rhizome has not been studied thoroughly yet, ignoring the fatal dose, toxic effects and side effects, thus, it is still widely used in medicinal wine. When taken improperly, poisoning may result due to overdose. Departments responsible for managing the marketing and supervision of such Chinese medicinal herbs fail to monitor this product closely and hence cases of overdose or accidental ingestion are quite

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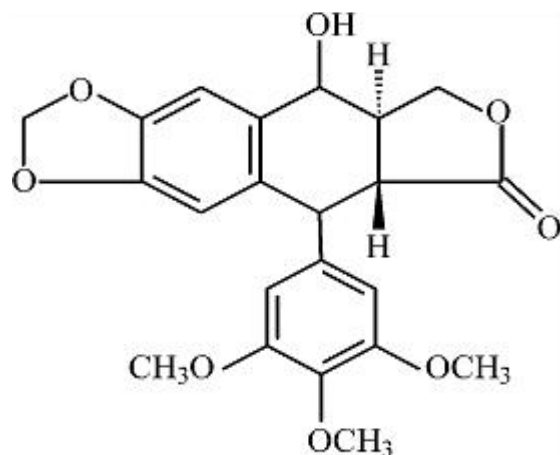


Figure 1. Structure of podophyllotoxin (Lv et al., 2006).

numerous (Feng et al., 2003; Jin and Yuan, 2003; Fang and Hong, 2003; Chen, 2004; Li and Jiang, 2007; Gong et al., 2007).

TOXICOLOGICAL EFFECTS

Effect on the skin

D. versipallis rhizome can induce skin dermatitis after contact because of acrimony; even death can occur if a large amount is involved. Song et al. (2001) and Gong et al., (2007) reported that patients who applied podophyllotoxin (major constituent of *D. versipallis* rhizome) ointment to cure severe condyloma acuminata were poisoned to death.

Effect on central nervous system

D. versipallis rhizome causes an excitatory followed by inhibition effect on the central nervous system. Brain damage occurs especially in the cerebral cortex, basal ganglia and spinal cord. In the peripheral nervous system, sensory nerve and motor nerve conduction velocity can be slightly or moderately reduced, and the distal latency period can be prolonged. Change in wave amplitude becomes obvious and the motor (nerve) conduction velocity (MCV) cannot be elicited in some areas. Electromyogram (EMG) can give strong positive results, and 'spontaneous potentials' are commonly observed. The motor unit potential (MUP) time is usually lengthened during mild contraction, but MUP can also be undetected in some regions. The uptake of MUP decreases during severe contraction and muscles innervated by damaged nerves can display simple phase. Muscle atrophy is obvious in the damaged sites, and muscle strength diminishes and tendon reflex becomes

weaker or disappears. This indicates that the affected nerves mainly show axonal injury and demyelination change is milder, and sensory nerve damage is more pronounced than motor nerve damage (Chen, 2004). JIN (Jin and Yuan, 2003) reported a case of toxic encephalopathy caused by mistakenly drinking a medicinal wine containing *dioscorea versipallis* rhizome. The clinical presentation was diffuse CNS intoxication. In this case, brain cells were damaged by podophyllotoxin (CT scan showed lentiform nucleus pathological change) and cerebral edema and coma resulted. The reason is that podophyllotoxin is able to cross the blood-brain barrier due to its high liposolubility.

Effect on digestive system

D. versipallis rhizome can cause severe gastrointestinal symptoms during the early phase of poisoning. Gastrointestinal tract peristalsis is strongly stimulated and absorbing function is dysregulated. The clinical symptoms are nausea, vomiting, stomach ache, diarrhea (Chang et al., 1992; Feng et al., 2003).

Effect on cardiovascular system

Cardiac muscles can be severely damaged by *D. versipallis* rhizome and lead to toxic myocarditis. The clinical symptoms include palpitation, chest pain, tachycardia, elevated cardiac enzymes. CHEN (Chen, 2004) reported a case of death by atrial fibrillation and coagulopathy involving intoxication by *D. versipallis* rhizome. The clinical presentations of the deceased were clearly related to cardiac toxicity, for example, fast atrial fibrillation, acute left heart failure and later cardiac arrest. Cerebral hernia and hyperkalemia were responsible for these signs and symptoms.

Cytotoxicity

The main toxic mechanism of podophyllotoxin (major constituent of *D. versipallis* rhizome) is cytotoxicity: (1) podophyllotoxin inhibits polymerization of cellular microtubules, which can affect the assembly of microtubules. This can lead to cessation of cell division at metaphase and also stop mitosis. (2) podophyllotoxin is a specific cell-cycle toxic agent which inhibits the activity of type II topoisomerase at the G₂ phase and S phase of a cell cycle. This creates a stable 'DNA- type II-topoisomerase-drug molecule' and induces the breaking of the double strand or single strand of DNA molecule, which in the end leads to illegitimate recombination DNA. (3) it inhibits cellular uptake of nucleosides such as thymine, uracil, adenine and guanine and hence, inhibit synthesis of DNA, RNA and protein (Imbert, 1998;



Figure 2. *Dysosma versipallidis* rhizome (root), in Shennongjia.

Xiao, 2002; Xia, 2005; Wang, 2006).

POISONING SYMPTOMS

Clinical symptoms of mild *D. versipallidis* rhizome poisoning includes lip numbness, dizziness, nausea, vomiting, abdominal pain, watery stool. Symptoms of severe poisoning are primarily digestive tract symptoms, and later cerebral nervous system symptoms, including dizziness, hypersomnia, coma, obnubilation, miosis, and tongue twitching, cyanosis of lips, conjunctival edema, ulcers of tongue and oral mucosa, and few moist rales heard at the lung base (Yang et al., 1994). Later, multi-organ function disorder, respiratory and circulatory failure, disappearance of reflexes, and cardiac arrest lead to death (Miao et al., 2007; Zheng, 2004). A case of multiple organs function failure due to oral overdose of dysosma versipallidis rhizome was reported. It described severe gastrointestinal symptoms, damage of liver function, and failure of the nervous system, hematological system, and circulatory system (Fang and Hong, 2003).

PATHOLOGIC CHANGES

Animal experiments showed that, when adult male rats were injected with podophyllotoxin (major bioactive constituent of *D. versipallidis* rhizome) at a dose of 10 or 15 mg/kg, they exhibited symptoms of nervous system poisoning. The pathologic examination showed a decreased number of Nissl bodies in the posterior column of the spinal cord and that the pathological change persisted for a rather longer period after intoxication. And that the axons of the peripheral nerve fibers were severely damaged. Most motor neurons of the anterior column of the spinal cord appeared edematous, but no necrosis was present (Chang et al., 1992). The results imply that intoxication by *D. versipallidis* rhizome not only causes metabolic dysfunction of the neurons but also

reduces the synthesis of soluble axoplasm, microtubules and neurofilament around the neuronal nuclei. This causes a change in the transportation ability of the axoplasm, and aggravates the extent of axonal degeneration (Louis, 1995).

ANALYSIS OF DYSOSMA VERSIPALLIS RHIZOME

Morphologic methods

Morphologic identification of *D. versipallidis* rhizome could be a preliminary screening in identification. There are several oblate tuberosities connected on the surface of *D. versipallidis* rhizomes, each with depressed caudex trace (Guo, 1992) (Figure 2). The morphologic evidence, including a couple of depressed disc-forms caudex trace and microscopic views of transverse section of rhizome and powder would be foundations which support the identification.

Instrumental analysis methods

Thin layer chromatography (TLC)

Huang et al. (1997) analyzed *D. versipallidis* rhizome solution extracts by TLC and ultraviolet lamp at wavelength of UV254 nm, UV365 nm or treatment of iodine vapor, $AlCl_3$ ethanol solution or phosphomolybdic acid solution. The sensitivity of the method is quite high and the reproducibility is excellent; thus it could be used for identification of major constituent. Samples were dealt with chromogenic agent (20% nitric acid solution of 2.5% ammonium cerous sulfate) after expansion with TLC; samples containing *D. versipallidis* rhizome presented brown spots. This method is practical and the analysis is rapid. It is an ideal qualitative analysis method (Zhu et al., 2005; Liang et al., 2009).

High performance liquid chromatography (HPLC)

HPLC is a significant instrumental analysis method, with great separation efficiency and analytic effectiveness for Chinese herbs such as *D. versipallidis* rhizome. Shu et al. (2000) put forward the fingerprint map of the *Dysosma* rhizome, having podophyllotoxin and its derivatives as analytic counterparts. The analysis turned the quantitation of a single valid index to a fingerprinting analysis of multiple active constituents and thus satisfied the multiterm identification requests. Yu (1999) carried out further research on quantification and analysis of the unknown constituents of podophyllotoxin and hence completed the analysis of the latter substance. Gao et al. (2004), with a mixture of ether and methylene dichloride ($V_{\text{aether}}: V_{\text{methylene dichloride}} = 3:1$), eliminated protein components from blood serum of animal models. He

thus, greatly excluded contamination in biological samples and eventually contributed to a high recovery rate of the HPLC method. Also, this method is very precise, and it can be used for pre-processing and can be valuable when analyzing biological samples from poisoning cases due to podophyllotoxin.

MEDICAL MANAGEMENT

No specific pharmacological treatment is currently available to treat *D. versipallis* rhizome intoxications. All therapies are symptomatic, including fluid and electrolyte replacement, cardiovascular and respiratory support, seizure control and conventional therapeutic methods for severe multiple systems failure. Some similar therapeutic approaches recommend that standard therapeutic practice should include induction of vomiting, bowel evacuation, gastric lavage and administration of activated charcoal.

In an attempt to develop better pharmacological treatments for *D. versipallis* rhizome intoxications, several compounds were utilized. Feng and Cai (2003) showed the inefficacy of ATP, coenzyme A, vitamin C, hormones, hemodialysis and cerebral protective agents to a poisoned man. In later cases, medicines aforementioned and intensive rehabilitation programme were applied. This treatment merely corrected amyotrophy and nerve conduction block but it failed to relieve much. Li and Jiang (2007) induced acupuncture for an intoxicated man and it helped to recover language function, sensory system and muscular strength and endurance. For patients whose symptoms displayed coagulopathy and atrial fibrillation, Chen (2004) and Zheng (2004) applied concentrated platelet suspension and intravenous immunoglobulin for the symptomatic treatment.

TOXICOLOGIC IDENTIFICATION

Questions remain besides the medical management, the difficulties in links between supervision, preparation (paozhi), application, doses and medicolegal issues. However, these questions are not insurmountable and will be addressed in future. Further, the very real dangers of having dysosma versipallis rhizome (including internal administration and external treatment) deserve acknowledgement. The public must acknowledge the toxicity of *D. versipallis* rhizome. Both its over-the counter sale and private sale should be restricted. A wide variation in the practice of this and other traditional Chinese medicine call into question the idea that there are universally agreed on criteria for choosing procedures based on arrived treatments. In the current culture that calls for certainty and reductionist thought, it is believed that the nearest thing to what is considered an objective body of theoretical and clinical knowledge is found deep within

the Chinese classic tradition.

A holistic approach must be observed when dealing with cases of intoxication due to *D. versipallis* rhizome, since the therapeutic dose and toxic dose levels are very close. And also, because of differences in individual body constitution, age etc, many situations deserve particular attention, including linking cases of accidental ingestion and overdose. Besides of injury, primary diseases and collecting samples of *D. versipallis* rhizome or other herbal drugs and hence perform a complete toxicological analysis. Careful autopsy and histopathologic examination of tissues should be done and particular attention should be paid to the pathological changes. Pathological changes and relevant mechanisms for acute and chronic poisoning due to *D. versipallis* rhizome has not been identified yet; this can influence medicolegal expertise of *D. versipallis* rhizome poisoning to a certain degree. Hence, further research on *D. versipallis* rhizome related poisoning is an important issue in toxicology and deserves urgent attention.

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