

Advances in the Safety Evaluation of Mineral Medicines - Cinnabar and Realgar

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Abstract

Mineral medicines have a long history in the treatment of diseases. Among these, cinnabar and realgar are the most commonly used. This review discusses the differences between cinnabar and realgar and other mercurials and arsenicals. In addition, it explores the toxicity studies carried out on cinnabar and realgar, which include acute and chronic toxicity, neurotoxicological effects, special toxicity, disposition and accumulation, and the relative mechanism of toxicity. Cinnabar and realgar are seldom used alone but rather in combination with herbs or animal medicines. Thus, in this article, we have also included a study of the effect of toxicity reduction and efficacy enhancement after cinnabar and realgar were combined with other Chinese medicinal materials. This review provides the theoretical evidence for their clinical application, suggesting that the dosage and treatment period using cinnabar and realgar should be rigidly controlled and emphasis should be placed on the drug safety of special populations.

Keywords: Cinnabar, compound Chinese medicines, disposition, realgar, toxicity

INTRODUCTION

Mineral medicines have been documented in many ancient codes and records^[1] and have been used in China for thousands of years. Forty-six mineral medicines, including cinnabar, realgar, limonite, mirabilite, and talc, etc., have been recorded in the *Sheng Nong Herbal Classic*, one of the four classics of traditional Chinese medicine (TCM) and the earliest books of the Chinese materia medica (CMM). In the subsequent CMM books, the types of mineral medicines have increased up to 222 mineral medicines, which were included in the *Compendium of Materia Medica*.^[2] Cinnabar and realgar were the most commonly used mineral medicines in CMM. In the 2015 edition of the *Chinese Pharmacopoeia*, approximately 7% of the Chinese patent medicines contain realgar or cinnabar, or both,^[3] in the prescription. Considering the frequent application of mineral medicines, the safety of cinnabar and realgar has been receiving extensive attention from the public.

calming the nerves, clearing away the heart-fire, and relieving convulsions.^[3] It has been used in the treatment of palpitations, infantile convulsions, insomnia or dreamy, epilepsy, infantile convulsions, faint vision, aphtha, throat obstruction, sores, and swellings, etc., Modern pharmacological studies have revealed that cinnabar possesses anxiolytic effects in association with a decline in the levels of serotonin 5-hydroxytryptamine (5-HT) in the brain, apart from those relevant to the 5-HT metabolism pathway.^[4] Cinnabar has been frequently used as an ingredient

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THE GENERAL SITUATION OF CINNABAR AND REALGAR

Cinnabar (Zhusha), also known as mercuric sulfide (HgS), is known to have a variety of functions, including detoxification,

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in CMM, of which over 40 are included in the 2015 edition of the *Chinese Pharmacopoeia*.^[3] In the early history of TCM, the use of cinnabar had a mixed response, with uncertainty about whether it should be considered nontoxic or toxic. Cinnabar is virtually insoluble in water and gastrointestinal fluid; hence, its absorption into the body is difficult, and it generally exhibits low levels of toxicity. Thus, it was listed as the foremost class of CMM in the *Sheng Nong's Herbal Classic*. This indicated that cinnabar was beneficial for the human body and had little or no toxicity.^[5] Subsequently, ancient TCM practitioners discovered that cinnabar could be converted into mercury, a toxic heavy metal element, on heating or burning. This was described in the *Compendium of Materia Medica* in the Min dynasty. Based on this understanding, cinnabar was recorded as a toxic mineral medicine in the subsequent ancient CMM books, such as the *Yao Xing Lun* (Tang dynasty) and the *Wu Pu Bencao* (Wei dynasty). Neurotoxicity due to the long-term use of cinnabar was first recognized in the Qing dynasty. In addition, in the *Essentials of Materia Medica*, cinnabar was described as being the cause of dementia on the long-term use.^[6]

Realgar, an arsenic sulfide mineral (also known as Xiong Huang), was first recorded in the *Shen Nong's Herbal Classic* as a CMM. Since then, it has been used for over 2000 years. It has been known to possess functions such as detoxification, the killing of insects and parasites, eliminating dampness and phlegm in the body, and antimalaria, among others. In addition, it can be used for the treatment of carbuncle, furuncle, snake bite, abdominal pain, epilepsy, and malaria (2015 edition of the *Chinese Pharmacopoeia*).^[3] Besides, realgar had been developed to treat malignant tumors and nosohemia.^[7] Wang et al.^[8] revealed that the application of realgar and *Indigo naturalis* in combination had therapeutic effects for promyelocytic leukemia. Some experimental and clinical observations implied the optimistic prospects of developing realgar as an anticancer candidate. However, since realgar is poorly soluble in water, researchers have now begun to focus on the development of new formulations.^[9] The nanometer-sized realgar displayed antitumor effects on the metastasis of MCF-7 cells (breast cancer) and A549 cells (lung cancer).^[10,11] Song et al.^[12] observed that the realgar-transforming solution had potential anticancer effects on human hepatocellular carcinoma (HepG2 cells) through the induction of ROS. Realgar is one of the most frequently used mineral drugs in CMMs. In the 2015 edition of the *Chinese Pharmacopoeia*, realgar was included in the prescriptions of 22 Chinese patent medicines.^[3,13] From ancient times, the toxicity of realgar was well-known. Currently, crude realgar is one of the 28 books of CMM listed in the catalogs of toxic medicinal materials that must be strictly controlled for use in China, according to the "Measures for the Control of Poisonous Drugs for Medical Use."^[14] However, it has been frequently used to ward off the insects and snakes because of its repellent properties, as well as being in TCM to relieve toxicity and kill parasites.^[15]

CHEMICAL FORMS OF CINNABAR, REALGAR, AND OTHER MERCURIAL AND ARSENICALS

Cinnabar contains over 96% mercuric sulfide (HgS).^[16] Realgar is a naturally occurring arsenic sulfide, containing >90% Tetra-arsenic tetra-sulfide (As₄S₄).^[17] However, different mercuric or arsenic compounds exhibit great variations in their chemical properties, especially with regard to toxicity.^[18] Among mercuric compounds, mercury chloride (HgCl₂), mercury vapor, and organic mercury such as methylmercury (MeHg) and ethylmercury (EtHg) are highly toxic. As for arsenic compounds, toxic inorganic arsenic compounds, including sodium arsenite (As³⁺) and sodium arsenate (As⁵⁺), can be transformed in the body to organic arsenic compounds such as monomethylarsonous acid, dimethylarsinic acid, and trimethyl arsenic acid.^[15] This warrants that the toxicity be largely reduced. Therefore, although mercuric or arsenic compounds contain similar metallic elements (mercury and arsenic), they have obvious differences in their chemical constitution, which could be major determinants of disposition and toxicity.

The physicochemical properties of cinnabar are completely different from those of other mercury compounds.^[19] The solubility and bioavailability of cinnabar are very low, making absorption into the body very difficult. A study compared the accumulation of mercury in the organs (OMAs) after the administration of similar doses of either cinnabar or mercuric chloride to mice. The results revealed that OMA after the administration of cinnabar was much less than that by mercuric chloride.^[20,21] Another report revealed that the oral administration of methylmercury to mice or rats could produce a large OMA, which is 1000-fold higher than that produced by cinnabar. The bioavailability of cinnabar is much lower than that of other mercury compounds, which is still about 1/30th to 1/60th of that of mercuric chloride.^[22] In addition, it is about 1/1000th of the bioavailability of methylmercury (in the aspect of producing neurotoxic effects).^[23] It is generally recognized that drug accumulation and bioavailability are pivotal determinants of toxicological effects. Thus, unsurprisingly, cinnabar exhibits significant differences in the toxicological effects in comparison with other forms of mercury.^[19] However, mercury vapor is easily absorbed by the lungs, and the absorption rates of lungs can reach up to 80%. Heating of cinnabar can yield mercury vapor, which can induce local or systemic toxicity.^[19] Thus, cinnabar should be used in the form of an unheated powder. Alternatively, it should not be prepared using heating methods such as decoction or roasting.^[3]

Similarly, realgar (As₄S₄) is also insoluble and has a very low bioavailability. A previous study revealed that realgar, in comparison with other forms of arsenic compounds (As³⁺ or As⁵⁺), was poorly absorbed. Thus, this resulted in a low accumulation of arsenic in tissues and failure to cause any tissue damage.^[15,24] In contrast with realgar, 80% of other orally administered arsenicals are easily absorbed from the gastrointestinal tract resulting in high arsenic concentrations

in the plasma and different tissues.^[15] The toxicity of realgar is lower than that of other arsenic agents due to the great reduction in solubility and bioavailability in comparison with other arsenicals.^[25]

SAFETY OF CINNABAR AND REALGAR

A vast proportion of the published literature has reported that the toxicological effects of cinnabar and realgar are much less than mercurials and arsenicals, respectively. However, the safety of their use has drawn extensive public attention due to concerns regarding the presence of heavy metals in cinnabar and realgar.

Acute toxicity studies of cinnabar and realgar

A previous acute toxicity study reported that mice exhibited adequate tolerance to cinnabar. In addition, no toxicity reactions were observed after single oral doses of 24 g/kg of cinnabar (300 times the clinical equivalent dose).^[26] According to “A guide to the Globally Harmonized System of Classification and Labeling of Chemicals,”^[27] if the LD₅₀ of a compound is >5 g/kg, this compound is considered to be nontoxic. Cinnabar belongs to the class of commonly nontoxic and harmless materials (category 5). This, therefore, indicates that a single dose of cinnabar is relatively safe.

Similarly, it was observed that the toxicity of realgar was very low when it was orally administered only once. The oral LD₅₀ of realgar in mice was 20.5 g/kg (12812 times the clinically equivalent dose).^[28] This substance also belonged to the class of basically nontoxic compounds (category 5).^[27] In an *in vitro* cell experiment, it was revealed that the LC₅₀ for realgar (2000 μM) was much higher than that of arsenic trioxide (280 μM), arsenite (35 μM), and arsenate (400 μM).^[24] Therefore, the results suggested that a single administration of realgar is relatively safe when used within the dosage range mentioned in the pharmacopeia.^[3]

Chronic toxicity studies of cinnabar and realgar

Although cinnabar may not cause obvious acute toxicity, long-term use may increase the risk of accumulation of mercury and subsequent injury of the organs. It was reported that cinnabar, which contains soluble mercury (≤20 μg/g) induced pathological changes in the kidney after rats received successive doses of cinnabar (0.4 g/kg) for 4 weeks.^[26] Furthermore, when the dosage was doubled to 0.8 g/kg, administration of cinnabar resulted in both liver and kidney damage. When the treatment period spanned 8 weeks or longer, hepatotoxicity and nephrotoxicity were observed even in the rats administered lower doses of cinnabar (0.1 g/kg). In a repeat toxicity study of cinnabar, there were no observed adverse effect levels (NOAELs) after 4-week and 13-week administration of 0.1 g/kg and 0.05 g/kg, respectively. Thus, the long-term use of cinnabar could induce hepatotoxicity and nephrotoxicity. Accordingly, the dose and treatment period should be limited. Based on these results, it can be suggested that cinnabar, which contains soluble mercury (≤20 μg/g) could be used for treatment periods of <6 weeks at a dose of 0.05 g/d,

or for <2 weeks at a daily dose of 0.1 g. For more information, the active guidelines should be referred.^[29]

After administration of realgar for 6 weeks, histopathological changes such as fatty degeneration and inflammatory cell infiltration were observed in the liver and kidney in the 0.2 and 0.4 g/kg groups in rats.^[30,31] The results indicate that the long-term use of realgar could induce toxicity, especially in the major target organs, the liver and kidney. Lower doses of realgar could also cause the liver or kidney toxicity, or both, with a prolongation of the period of treatment. For instance, realgar (containing soluble arsenic 1.7 mg/g) could induce nephrotoxicity or hepatotoxicity after administration of 80 mg/kg or 160 mg/kg of realgar for >8 weeks, respectively. NOAELs of realgar for 4 weeks, 8 weeks, and 13 weeks of administration were 160 mg/kg, 20 mg/kg, and 10 mg/kg, respectively. In order to safely use realgar, it is suggested that under the conditions of ≤1.7 mg/g of soluble arsenic, realgar should be used for <2 weeks at a dose of 160 mg/day, <4 weeks at a dose of 20 mg/day, and <6 weeks at a dose of 10 mg/day.^[28] In this regard, it would be advisable to refer to the “long-term toxicity test of chemicals technique guideline.”^[29]

Neurotoxicological effects of cinnabar and realgar

As early as the Qing dynasty, there existed a statement that warned of the induction of dementia on long-term use of cinnabar.^[6] Modern pharmacology research has reported that the administration of cinnabar (1 g/kg) for 30 consecutive days could induce episodic memory behavior in the affected rats. These results revealed a decrease in the number of active avoidances and an increase in the latency and shock period.^[32] In addition, previous studies also reported that on exposure to cinnabar (1 g/kg) for 1 week, Hartley strain guinea pigs suffered from not only hearing impairment but also dysfunction of the vestibulo-ocular reflex system. This further resulted in learning and memory impairment.^[33] The spontaneous locomotor activities were preferentially suppressed, especially in male rats. The pentobarbital-induced sleeping time was prolonged, and the retention time on the rotating rod was reduced after the administration of cinnabar for 6 weeks. These effects progressively increased to a large extent as the treatment was increased to 11 weeks.^[34] The long-term administration of clinical doses of cinnabar (10 mg/kg/day) could induce ototoxicity in mice. In addition, it was observed that male mice were more sensitive to cinnabar in the development of hearing impairment.^[35]

Behavioral studies revealed that treatment with arsenic could cause a decrease in the locomotor activity and learning deficits in a delayed alternation task.^[36] However, only a few studies have investigated the neurotoxicity of realgar. After 6 weeks of exposure to realgar (2.7 g/kg), the rats exhibited significant deficits in spatial learning and memory ability, open field cognitive ability, as well as the ability to identify new things. This suggests that the long-term administration of realgar could result in neurotoxicity.^[37]

Special toxicity of cinnabar and realgar

Some Chinese patent medicines that are used in pediatrics contain cinnabar or realgar, or both, such as Baochi San, Yinianjin, and Xiao Er Jiere Wan. The proportion of pediatric drugs containing these two substances is shown in Figure 1.^[38] In addition, the contraindication of some Chinese patent medicines containing cinnabar and realgar during pregnancy was not clearly specified in the *Chinese Pharmacopoeia*.^[3] Moreover, there is little information about whether Chinese patent medicines containing cinnabar or realgar could affect children or pregnancy. To ensure the safe use of these Chinese medicines, more research is required about the special toxicity of cinnabar and realgar. This will provide a reference for the appropriate clinical selection of medicines.

Liang *et al.*^[39] reported that when cinnabar was administered to mice before pregnancy or during the first trimester of pregnancy, it could be harmful to the fetus at a dose of 0.08 g/kg/day. However, no obvious embryonic toxicity was observed when cinnabar was administered orally at the intermediate stage or during late pregnancy. These results suggest that the embryos in the early stages of pregnancy could be more sensitive to cinnabar than the other stages. Gu *et al.*^[40] reported that cinnabar had a toxic effect on the fertility of male rat as well as on early embryo implantation and development. With regard to the long-term administration of cinnabar, the absorbed mercury could pass through the placental barrier and accumulate in the embryo, inducing injury and poisoning.^[41]

Realgar exhibited effects such as potential mutations in PCE mouse marrow micronucleus assay at a dose of 1 g/kg.^[42] In addition, mice exposed to realgar during pregnancy may lead to the development of increased susceptibility to liver or lung cancers in the mice offspring.^[43] In animal experiments, the effect of realgar on embryos is somewhat controversial. A study used teratogenic methods (*in vivo*) combined with scanning

electron microscopy and histochemical methods to investigate the arsenic-induced developmental toxicity and the mechanism involved by an evaluation of the effects of arsenic on pregnant rats, fetuses, fetal cells, and so on.^[44] The results revealed that arsenic had certain teratogenic effects on rats. However, other studies concluded that the oral administration of realgar had no significant toxicity on fertility and early embryo development in rats.^[45] Pregnant rabbits were more sensitive to the toxicity of realgar than pregnant rats and manifested the occurrence of maternal toxicity. It was observed that the living fetus rate was decreased, and the late fetus loss rate was increased. However, the obvious teratogenic effect of realgar was not observed in pregnant rabbits.^[46] However, there has been no exact information about the effect of realgar on human embryos at a certain dose and time range so far. Arsenic compounds have been recognized by the WHO as carcinogens in humans. Epidemiological studies have revealed that arsenic may cause skin and lung cancers in human on long-term exposure. In a survey for lung cancer in 1981, researchers found a high risk of lung cancer among workers with high exposure to arsenic.^[47] There was a unique black-foot disease, which confined the endemic area to the southwestern coast in Taiwan in 1960.^[48] Researchers observed that this disease was related to long-term exposure to high doses of arsenic.^[49] A significant dose-effect relationship was observed between arsenic concentration in well water and the incidence of skin, kidney, lung, and bladder cancers in both males and females, and cancers of the liver and prostate in males.^[50] As an arsenic compound, realgar has also been reported to increase the risk of skin and lung cancer in those individuals who are exposed to realgar for long periods. It was reported that there were about 50 cases of malignant tumors occurring in the Hunan realgar mine between 1971 and 1981, including 35 cases of death. Among the 50 cases of malignant tumors, there were 22 cases of lung cancer, accounting for about 44% and ranking first.^[51] In addition, between 1974 and 1984, there were about 15 cases of skin cancer diagnosed in the area. However, so far, the cases of human cancers caused by realgar in the published literature are related to occupational long-term exposure or environmental pollution. There has not been a single report regarding the potential of the use of realgar-containing Chinese medicines to cause cancer.

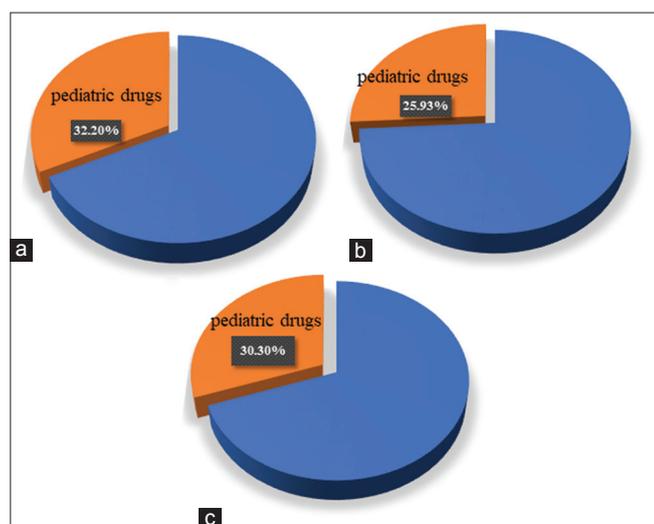


Figure 1: The proportion of pediatric drugs containing cinnabar or realgar, or both. (a) medicines only containing cinnabar; (b) medicines only containing realgar; (c) medicines containing both cinnabar and realgar

DISPOSITION OF CINNABAR AND REALGAR

Absorption

After powdered cinnabar was administered to mice for 5 days, <0.02% of the dose was measured in the kidney and liver tissues. Only about 0.2% of the dose of cinnabar was absorbed from the gastrointestinal tract.^[20] Thus, the bioavailability of cinnabar is much less than other mercuric compounds. Similarly, only 4% of arsenic in the form of realgar in the *Niu Huang Jie Du Pian* was available for absorption into the bloodstream.^[52] Over 82% was found in the feces after oral administration of realgar alone for 3 days.^[15] After oral administration of realgar in rats (150 mg/kg) for 5 weeks,

only a small portion of arsenic was absorbed and reached systemic circulation (45 mg/mL).^[15] However, nanoparticles of realgar exhibited a significant increase in the bioavailability in comparison with the traditional realgar powder. Urinary recovery of arsenic in rats after administration of realgar nanoparticles was increased to 70%, in comparison with the traditional realgar powder (25%).^[53]

Distribution and accumulation

The distribution of mercury from cinnabar followed a distribution pattern similar to other mercurial compounds. The mercury concentration in different organs after long-term use of cinnabar is as follows: kidney > liver > brain. Mercury accumulation from cinnabar in the liver (4.42 µg/g) was the equivalent to 20% of that in the kidneys (18.06 µg/g). Oral administration of cinnabar allowed its distribution into the brain (about 12% of renal accumulation), with amounts concentrated mainly in the cerebral cortex (1.17 µg/g) and the cerebellar cortex (1.04 µg/g).^[54]

After single dose administration of realgar at doses of 160 mg/kg, the distribution of arsenic in the organs from high to low concentrations was: blood > kidney > lung > liver > heart > brain. After repeated administration of realgar for 13 weeks, the main organs displayed arsenic accumulations at different extents, including the blood, kidneys, liver, and the brain. The highest arsenic amount of accumulation was found in the kidneys, followed by the liver.^[55] The content of arsenic accumulation in the tissues and organs was in the order of blood > kidney > liver > brain. It was obvious that the blood was the major target tissue due to the highest arsenic distribution and accumulation after realgar administration.^[56] This result could also provide the basis for the efficacy of realgar in the treatment of leukemia.

Elimination and excretion

The half-life $t_{1/2}$ was measured as 4.2 ± 0.5 h when the rats were intraperitoneally administered cinnabar at clinical doses of 50 mg/kg. About 45 µg/L mercury was detected in the feces of rats administered cinnabar for 12 h. In addition, a small amount of mercury was detected in the feces after cinnabar was administered for 96 h.^[57] It took a long time to eliminate the absorbed mercury from cinnabar and the excretion rate was slow. Thus, the long-term use of cinnabar might result in toxicity owing to the accumulation of mercury.

When rats were single orally administered realgar at a dose of 75 mg/kg for 96 h, the amount of fecal excretion accounted for 97.7% of the total intake of realgar. The amount of biliary excretion was 35.39% and arsenic excretion in the urine was only 0.0367%.^[58] There was a decrease in the total amount of arsenic excreted with an increase in the dose. These data suggest that an overdose of realgar might cause toxicity.

TOXICITY MECHANISM

The highest concentration of mercury observed in the kidneys could be associated with the high sensitivity of

the kidneys to mercury.^[59,60] This explains why the dose that induced nephrotoxicity was much lower than the dose at which hepatotoxicity was induced. The phenomenon of prolonged use of cinnabar inducing nephrotoxicity could be related to the apoptosis of the tubular cells through the death receptor-mediated pathway.^[61] In addition, it could be associated with the inhibition of several members of the organic anion transporters (OAT1 and OAT3).^[62] The proximal straight tubule is the segment of the nephron that is most vulnerable to the toxic effects of mercury. Therefore, this conclusion provides the theoretical basis for further research on the targets of cinnabar-induced nephrotoxicity. In addition, the long-term use of cinnabar may induce neurotoxicological effects even at relatively low doses. This could be related to the inhibition of the Na⁺-K⁺-ATPase activities and increasing NO levels.^[33,63]

The toxicological effects of realgar could be related to the oxidative damage induced by the activated reactive oxygen species.^[64] The NMR-PR analyzed results of the extracts in the serum, urine, and the liver highlight the complex disturbances in the profiles of endogenous metabolites, which could be induced by realgar. Exposure to realgar could affect energy metabolism, transmethylation, gut microflora environment, and amino acid metabolism.^[65] It can be suggested that realgar could cause the accumulation of triglycerides and free fatty acids, resulting in hepatotoxicity.^[66] Besides, the relatively high levels of accumulation of arsenic from realgar in the blood imply its potential hematotoxicity.^[67] Long-term exposure to realgar could induce an increase in the Glu concentrations in the synaptic cleft of the hippocampus, which can result in neuronal death, an increase in the intracellular calcium levels by an influx of calcium, and an increase in the expression of NR2A mRNA in the hippocampus. Further, pathological changes in the three key organelles (mitochondrial, rough endoplasmic reticulum, and the Golgi complex) in the hippocampal neurons were observed, which could affect the metabolic activity of the cells. The above could be the underlying mechanism of the neurotoxicological effect induced by realgar.^[37]

TRADITIONAL MEDICINES CONTAINING MAINLY CINNABAR OR REALGAR, OR BOTH

Some frequently used medicines containing cinnabar or realgar, or both, are listed in Table 1. Cinnabar and realgar are usually used in combination with herbal or animals' medicines rather than alone. This is probably to reduce the toxicity and enhance the efficacy.^[18] For example, a metabolic profiling analysis based on ¹H NMR spectroscopy was applied to investigate the protective effects of Zhusha Anshen Wan on the toxicity effects caused by cinnabar alone.^[16] Acute nephrotoxicity and hepatotoxicity of cinnabar were evident in rats orally administered a dose of 1.8 g/kg for 7 days. However, the toxicity of Zhusha Anshen Wan was mild or absent in rats administered a dose of 9 g/kg. Similarly, the results of another metabolic profiling analysis revealed that the other herbs in the

Table 1: The medicines containing cinnabar or realgar, or both

Medicine	Minerals included	Other ingredients	Pharmacological effects
Zhusha Anshen Wan	Cinnabar	<i>C. chinensis</i> Franch, <i>G. uralensis</i> Fisch, <i>R. glutinosa</i> Libosch, <i>A. sinensis</i> (Oliv) Diels	Sedating the heart, draining the fire, calming the spirit, and nourishing Yang ^[16]
Niu-Huang-Jie-Du Tablet	Realgar	Borneolum synthetium, <i>S. baicalensis</i> Georgi, <i>R. officinale</i> Bail, <i>G. uralensis</i> Fisch, <i>P. grandiflorum</i> (Jacq.) A.DC, etc.	Detoxification ^[68]
Huang-Dai Pian	Realgar	Indigo naturalis, <i>S. miltiorrhiza</i> Bge, <i>P. heterophylla</i> (Miq.) Pax	Acute promyelocytic leukemia ^[69]
An-Gong-Niu-Huang Wan	Both cinnabar and realgar	<i>C. chinensis</i> Franch, <i>S. baicalensis</i> Georgi, <i>G. jasminoides</i> Ellis, <i>C. wenyujin</i> Y.H.Chen and C.Ling, <i>P. martensii</i> (Ounker), etc.	Treatment of acute and chronic brain diseases, including cerebral stroke, viral encephalitis, severe cranio-cerebral trauma, and cerebral hemorrhage ^[70]
Hua-Feng Dan	Both cinnabar and realgar	<i>G. elata</i> Bl., <i>N. tenuifolia</i> , <i>A. lancea</i> (Thumb.) DC., Borax, Borneolum syntheticum, <i>B. martensii</i> Karsch (Quanxie), etc.	Hemiplegia, epilepsy, and facial paralysis

C. chinensis: *Coptis chinensis*, *G. uralensis*: *Glycyrrhiza uralensis*, *R. glutinosa*: *Rehmannia glutinosa*, *A. sinensis*: *Angelica sinensis*, *S. baicalensis*: *Scutellaria baicalensis*, *R. officinale*: *Rheum officinale*, *P. grandiflorum*: *Platycodon grandiflorum*, *S. miltiorrhiza*: *Salvia miltiorrhiza*, *P. heterophylla*: *Pseudostellaria heterophylla*, *S. baicalensis*: *Scutellaria baicalensis*, *G. jasminoides*: *Gardenia jasminoides*, *C. wenyujin*: *Curcuma wenyujin*, *P. martensii*: *Pteris martensii*, *G. elata*: *Gastrodia elata*, *N. tenuifolia*: *Nepeta tenuifolia*, *A. lancea*: *Atractylodes lancea*, *B. martensii*: *Buthus martensii*

Niu Huang Jiedu Tablet could have synergistic detoxification effects on realgar.^[71]

Decreasing the toxicity by the application of cinnabar and/or realgar in combination with other herbs and animal drugs could be associated with a decrease in the accumulation of mercury or arsenic. Compared to the administration of realgar alone, the t_{max} of realgar was significantly delayed when it was administered as the Niu Huang Jiedu Pian. In addition, there was a significant increase in the values of clearance and the apparent volume of distribution.^[72] In addition, the study based on 1H-NMR metabonomics revealed that the other chemical components in the Niu Huang Jiedu Pian could ameliorate the toxicity of realgar through the regulation of the energy, choline, and amino acid metabolism and modulation of gut flora.^[73] Besides, the effect of using Niu Huang Jiedu Pian to decrease the toxicity induced by realgar could also be associated with the inhibition of hepatocyte apoptosis through the signaling pathway of Bax/Bcl-2.^[74] Other components in the *Liushen* pills could decrease the toxicity of realgar by promoting the transition of arsenic from the high-toxicity form to low-toxicity form.^[75] Herbal medicines in the An-Gong-Niu-Huang Wan could attenuate the realgar-and cinnabar-induced hepatotoxicity and nephrotoxicity through an improvement of the antioxidant competence and suppression of the inflammatory injury.^[76] In addition, cinnabar and realgar processing in traditional medicines could be one of the methods used to reduce the toxicity. According to the 2015 edition of the *Chinese Pharmacopoeia*, cinnabar and realgar must be properly processed before they are used in medicine. The recommended processing method for both cinnabar and realgar is “Shui-fei” (finely grinding the medicinal material with water), which could contribute to the decreased toxicity through the removal of the soluble mercury and arsenic. The results of X-ray diffraction analysis revealed that the toxic form was removed after the purification of realgar by Shui-Fei.^[77]

However, the combination of cinnabar, realgar, and allopathic or western medicines could produce effects opposite to those exhibited by Chinese medicines. Cinnabar and its preparations combined with western medicines such as mist tribromide, ferric sulfates, potassium iodide, and sodium iodide could induce an increase in the toxicity as the mercury ions in cinnabar would be reduced to metallic mercury.^[78] Realgar and its preparations combined with western medicines such as glyceryl trinitrate and isoamyl nitrate could cause an increase in the toxicity as the nitrate radical in the latter could oxidize As_4S_4 in realgar.^[78]

DISCUSSION

The nonrational use of drugs is the primary cause of the toxicity induced by cinnabar and realgar

In the long-term toxicity studies of cinnabar and realgar, the safety of cinnabar and realgar was observed to be associated with the dose and the treatment period. In general, the use of cinnabar and realgar is relatively safe when lower doses are used or the period of treatment is shorter. Clinical studies have demonstrated that almost all adverse reactions were related to the irrational use of drugs, except for occasional allergic reactions.^[51] The dose of cinnabar observed in poison victims was usually a single administration of 10–60 g of cinnabar, which is equivalent to 20–120 times the daily maximum dosage mentioned in the pharmacopoeial regulations.^[3] On the other hand, the dose of realgar observed in poison victims was a single administration of 6–20 g, which is equivalent to 60–200 times the daily maximum dose mentioned in the pharmacopoeial regulations.^[3] Besides, with regard to cinnabar and realgar, poisoning in many cases was induced by drug accumulation due to repeated administration, while in other cases it could be an overdose. In most cases, it could be suggested that the poison victims were taking the medication for more than a month with a subsequent overdose as well.^[79]

Cinnabar and realgar are drugs, not food. However, these medicines are easily available in grocery stores or pharmacies

and are considered “dietary supplements.”^[80] For example, there was a case of a patient who presented with mercury poisoning because she ate pork heart cooked along with cinnabar. This led to an increase in the ingestion of soluble mercury by the patient.^[81] In addition, another patient suffered from arsenic poisoning due to the ingestion home-made realgar wine for years. There was still another case wherein an 87-year-old male inhaled the vapors of cinnabar intended to treat foot ulceration. Subsequently, he developed an acute lung injury and eventually died from profound hypoxemia.^[82] Therefore, abuse of cinnabar and realgar after disobeying the advice of doctors could be a potential risk due to the induced toxicity. It should be kept in mind that one should consult the relevant guidelines and pharmacopoeias before using these medicines.

Contraindications in pregnant women, newborns, and children

The studies of animal experiments showed embryonic toxicity were observed when cinnabar was administered in early pregnancy period. Besides, maternal toxicity and a decrease in the living fetus rate were observed in pregnant rabbits administered realgar. According to the 2015 edition of the *Chinese Pharmacopoeia*, use of cinnabar and realgar is forbidden in pregnant women. Further, young children are more sensitive to cinnabar and realgar and therefore more prone to poisoning and even death. It was reported that 10 newborns showed different degrees of toxicity symptoms, with the subsequent death of two children, after they were administered cinnabar for 5–7 days at an average dose of 6.3 g.^[83] Another report revealed that 11 children suffered from liver damage after they were administered Qianji San (containing cinnabar) for 3–7 days.^[84] The use of realgar in children is similar to cinnabar. It was reported that a child suffered from arsenic poisoning and subsequently died after the external application of realgar for 8 days.^[85] However, during our review of the published literature, we came across some prescription drugs containing cinnabar or realgar, or both, for pediatric use. In the *Chinese Pharmacopoeia*, there were 66 kinds of medicines containing realgar and cinnabar, including 20 for pediatric use. Furthermore, there were 27 kinds of drugs containing realgar alone, including seven for pediatric use. Further, there were 59 kinds of drugs containing cinnabar alone, including 19 for pediatric use. The dosage of seven kinds of children’s drugs containing cinnabar alone exceeded the adult dose by >50%.^[38] Since the gastrointestinal tracts of children have a greater propensity for absorption, especially of drugs such as mercury and arsenic,^[69] and the development of the detoxification and excretion functions of the liver, kidneys, and nervous system are not perfect, cinnabar and realgar should be used with caution.

Precautions in patients with liver or kidney dysfunction

A large number of studies have shown that the accumulation of mercury and arsenic in the liver and kidneys was more than that in other organs or tissues. There could be a positive correlation

between the degree of accumulation and liver and kidney damage. Therefore, overdose or long-term use of cinnabar and realgar could cause hepatotoxicity and nephrotoxicity.^[86] For risk avoidance and according to the 2015 edition of the *Chinese Pharmacopoeia*, the patients with liver or kidney dysfunction are forbidden to use cinnabar while realgar should be used with caution.

Reducing the toxicity of cinnabar and realgar by processing and combination use

In animal experiments, it was observed that the use of Chinese patent medicines (such as the Niu Huang Jie Du Tablet, the An-Gong-Niu-Huang Wan, the Liushen Wan, etc.) was safer and much less toxic than the use of cinnabar and realgar alone. However, they are not absolutely safe for clinical use. Take the Niu Huang Jie Du Tablet, for example, its administration usually induces acute adverse reactions, including anaphylactic shock and cutaneous anaphylaxis. On the other hand, chronic adverse reactions observed on prolonged use include liver injury, dermatitis, cystitis, and urocystitis. The major reasons related to these adverse reactions are its long-term use, overdose, and the poor quality of these drugs.^[87] An individual who was administered the Niu Huang Jie Du Tablet at three times the clinical dose for 4 years was ultimately diagnosed with the liver and kidney injury.^[71]

Mechanism of hepatotoxicity, nephrotoxicity, and neurotoxicity induced by cinnabar and realgar

The mechanism of the toxicity of cinnabar and realgar is mainly concentrated in the oxidative damage and induction of apoptosis. The mechanism of neurotoxicity induced by cinnabar and realgar is related to their effects on the enzymatic activity, Glu concentrations, and calcium levels. Most studies that have been conducted on the mechanism are generally insufficient. In addition, with the development of metabolomics research, several liquid chromatography-mass spectrometry (MS)-, gas chromatography-mass spectrometry/MS-, and NMR-based metabolomics analyses have been applied to investigate the detoxification of compound compatibility and the toxicity effects induced by cinnabar and realgar. This novel analysis offers an ideal platform for further investigation of the mechanism of toxicity and the assessment of the safety of cinnabar and realgar.

CONCLUSION

It can be suggested that close attention should be paid to the usage of cinnabar and realgar, and their preparations. There should be rigid controls on the dose and period of treatment, which can assist in decreasing the toxicity induced by overdose or long-term use. In addition, the rational use of cinnabar and realgar should be encouraged, with warnings to avoid abuse and self-medication. Further, pediatric drugs containing cinnabar or realgar, or both, should be used with caution. Moreover, there should be constant monitoring of the liver and renal functions after the administration of these drugs in patients with renal or hepatic insufficiency. Finally, patients should try to use cinnabar and realgar only after appropriate

processing and avoid combining them with western medicines, which could result in an increase in the toxicity.

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Conflicts of interest

There are no conflicts of interest.

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