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Beneficial and adverse effects of toad venom, a traditional Oriental medicine

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SUMMARY

Toad venom, "Chan su" in Chinese and "somso" in Korean, is a well-known traditional oriental medicine obtained from the skin venom gland of the toad. Formulations of toad venom have been widely applied in China, Japan, Korea and other oriental countries for a long time. It is often found in traditional Chinese formulations, such as Jiuxin (or Kyushin in Japan), Yixin, Huoxin, Shexiang baoxin wan, Lu shen wan and Laryngitis pills. According to a pharmaceutical chemistry study, toad venom contains multiple biological active substances, such as bufalin, resibufogenin and cinobufagin. Modern pharmacological studies indicated that toad venom has multiple pharmacological actions, including acting as a cardiotonic, antitumor local anesthetic effects, stimulates the respiratory center, vasopressor action, anti-inflammatory and diuretic effects. Like other medications, toad venom also has certain toxicity and adverse effects, for example, inducing delayed afterdepolarization and triggered arrhythmia. The major chemical constituents, basic pharmacological actions and adverse reactions of toad venom are discussed in this article.

Key words: Toad venom; Chemical constituents; Pharmacological actions; Adverse reactions

INTRODUCTION

Many amphibians produce irritating and unpleasant skin secretions that, in some cases at least, provide partial defense against predation; compounds isolated from such amphibians often possess remarkable pharmacological and toxical activities (Daly and Myers, 1967). Toad is an amphibian animal and its secretion or production, toad venom, is a regarded compound. Toad venom (dried secretion of the skin glands of *Bufo bufo gargarizans* Cantor of *B. melanotictus*) is obtained from the dried skin glandular secretary product and contains multiple biological active substances. Toad venom is referred to secrete bufonis in pharmacology and in China, toad venom is called Chan su, a Mandarin name. Chan su translates to Senso in Japanese and Somso in Korean. Chan su has been known in Chinese medicine for centuries. Toad venom is found in various traditional Chinese formulations. There are dozens of pharmacological preparation forms of toad venom in China: table, pill, power, injection, liquid, oil, ointment, tincture, cinnabar and lozenge, *etc* (Cheng, 2001). These traditional medicine formulations and their products have been widely used in China, Japan, Korea and other Asian countries (Henderson et al, 1962), as well as imported into the U.S. and Europe.

There is a long history for application of toad venom all over the world. Very early on, the toad was known as a venomous animal. In the Talmud under the name of *tzab*, it is differentiated from the frog and is classed with animals whose touch contaminates. Historically, various peoples have made medicinal use of the toad. The Chinese have long used a preparation derived from toad skin. According to a famous herbal medicine book in China "Pentsao Kang Mu" (1596) by Li SC, the drug can be employed by external application in the treatment of canker sores, toothache, sinusitis,

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and many local inflammatory conditions, and in the arrest of hemorrhages from the gums. However, toad venom, an impure product, is similar in its action to digitalis, but 50 to 100 times more powerful (Abel and Macht, 1911; Shimizu, 1916; Chen *et al.*, 1931; Chen *et al.*, 1933; Chen and Kovarikova, 1967). Western nations made use of the toad for medicinal purposes for several centuries and various European medical treatises and pharmacopeias of an earlier day give the dried toad a prominent place among therapeutic agents (Abel and Macht, 1911).

Though toad venom was used as an agent in the Orient for much longer, real scientific study occurred in Western countries. In 1912, Abel and Macht obtained bufagin ($C_{18}H_{24}O_4$) from tropical toad by scientific methods. They used physiological techniques to demonstrate that bufagin produced great increases in tonicity and contractility of the heart, as well as a slowing of the heart beat. They also found evidence of an effect on the His bundle of heart, causing a disturbance in conductivity and resulting in heart-block. On the other hand, the side effect could have been brought on by the toxicity of toad venom, expressed by high dose administration (Chen *et al.*, 1931; Chen and Kovarikova, 1967).

MAJOR CHEMICAL CONSTITUENTS

There are more than 250 species of *Bufo bufo* in the world and dozens of substances have been isolated from toad venom. It contains several potent substances, which are genin derivatives and alkaloids (Figure 1). The cardiac genin group includes bufalin ($C_{24}H_{34}O_4$, (3b, 5b, 14b)-3,14-dihydroxy-5-bufa-20,22-dienolide), cinobufagin ($C_{26}H_{34}O_6$, (3b,5b,15b, 16b)-14,15-epoxy-3,16-dihydroxy-5-bufa-20,22-dienolide-16-acetate), resibufogenin ($C_{24}H_{32}O_4$, (3b,5b,15b)-14,15-epoxy-3-hydroxy-5-bufa-20,22-dienolide), bufotalin ($C_{26}H_{36}O_6$), telocinobufagin ($C_{24}H_{32}O_6$), cinobufotalin ($C_{26}H_{34}O_7$), resibufogin ($C_{24}H_{30}O_3$), and bufotalinin and so on. The alkaloid group has bufotenine and bufotenidine. The structure of resibufogenin, bufalin and cinobufagin are similar to that of other digitalis glycosides. The chemical structures of several cardiac genins and digitoxigenin are shown Fig. 1. Based on toxic action, these preparations of toad

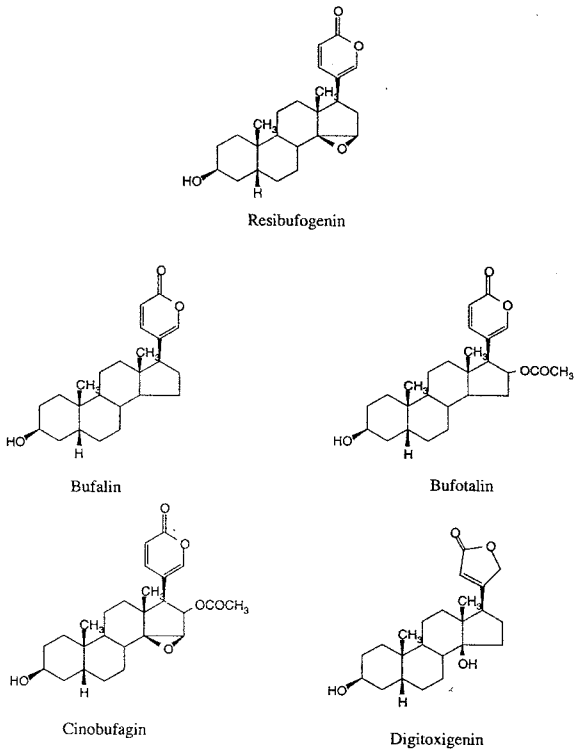

Fig. 1. The chemical structures of the major ingredients of toad venom and digitoxigenin.

venom contain two groups of toxic compounds. 1) Steriod derivatives that resemble cardiac glycosides consist of bufagenin and bufotoxins. 2) The basic components include epinephrine, norepinephrine, serotonin and bufotenine (Ko *et al.*, 1996).

Resibufogenin and bufalin are separated and extracted from toad venom. The isolative procedure of resibufogenin and bufalin is shown in Fig. 2 (Takagi and Ito, 1979).

BASIC PHARMACOLOGICAL ACTIONS

Previous reports indicated that the toad venom possesses multiple pharmacological actions

1) *Cardiotonic.* Resibufogenin, bufalin and gamabufotalin can increase myocardial contractility and improve circulation. Resibufogenin significantly increased ventricular contractile force in animal experiments, for example, increasing 50% ventricular contractile force in adult mongrel dogs, 36% in adult mongrel cat, and 34% in rabbits (Table 1). Isolated heart experiments also demonstrated that resibufogenin (0.1 mg/kg) increased rabbit isolated

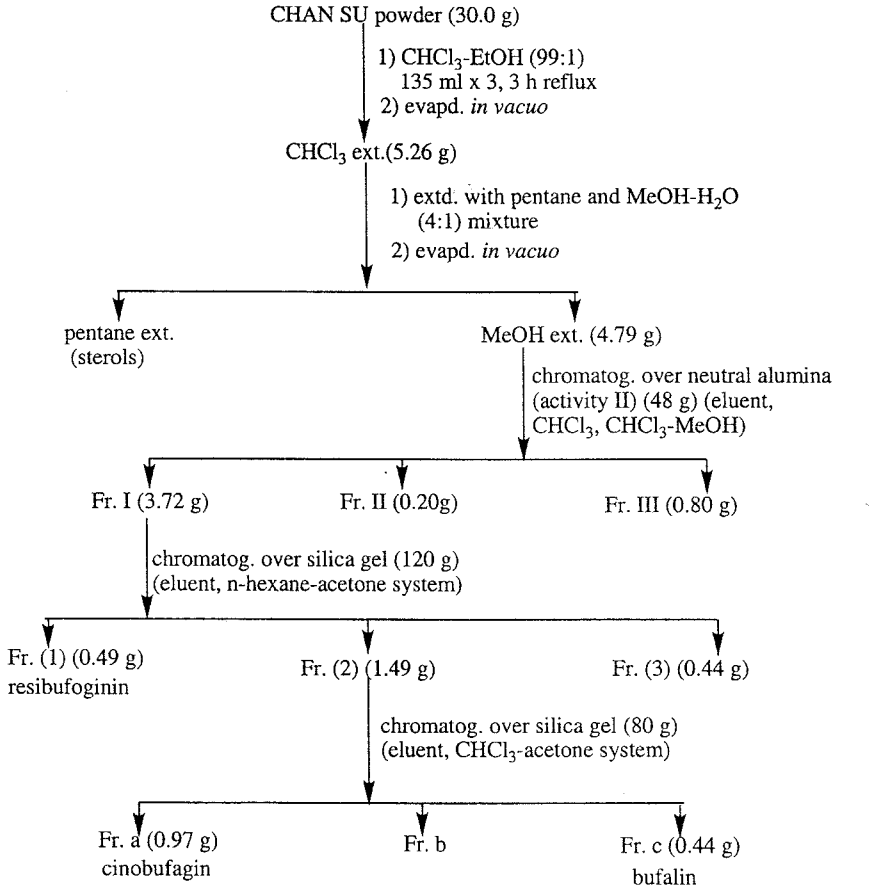

Fig. 2. The isolative procedure of resibufogenin and bufalin from toad venom.

heart contractile force. The maximum increasing percentage was 59% (Abel and Macht 1911; Okada *et al.*, 1960; Iwatsuki *et al.*, 1965; Xie, 1987; Xie *et al.*, 1975, 1977; 1993; 2000; 2001). Several lines of studies showed that the crude venom from the parotid gland of *Bufa marinus* toad increased myocardial contractility (Morishita *et al.*, 1992; Bagrov *et al.*, 1993; Huang, 1999; Cheng, 2001).

2) *Local anesthetic.* Bufalin, cinobufagin and bufotalin have a potent surface anesthetic effect. In local anesthetic potency on the rabbit cornea, bufalin was the most potent; its local anesthetic potency was about 90 times stronger than cocaine and its duration of action is relatively long. The other experiments indicated that cinobufagin in 0.1-0.5% solutions produces numbness of the tongue when applied locally. With the stronger solution, sweet and bitter tastes senses may be lost. It is suggested that the local anesthetic action is not on nerve fibers, but is confined to sensory nerve endings. In term of chemical structure, the significance of the presence of unsaturated lactone

Table 1. Effect of resibufogenin (0.2 mg/kg, i.v.) on heart contractile force.

Animals	N	Heart contractile force increase (%)				
		1′	3′	5′	10′	30′
Rabbits	12	27±2.3*	34±3.0*	23±2.0*	25±1.9*	21±1.7*
Cats	4	1±1.5	36±3.3*	36±3.3*	27±2.5**	---
Dogs	4	14±0.8	32±2.8*	22±1.8**	26±2.4**	---

*: $P<0.01$; **: $P<0.05$

ring, besides that of hydroxyl in C_3-position of the steroid ring, seems to be essential for the local anesthetic action (Okada and Ishihara, 1958; Okada et al, 1960; Okada, 1966).

3) *Stimulates the respiratory center*. Uniquely, the specific pharmacological effect of resibufogenin is a stimulatory action on respiratory system (Abel and Macht, 1912; Okada *et al.*, 1960; Iwatsuki *et al.*, 1965; Suga, 1973; Xie *et al.*, 1975; 1977; Morishita *et al.*, 1992). We observed respiratory stimulant effects of resibufogenin in rabbit experiments *in vivo*. Respiratory amplitude, tidal volume, and minute volume increased significantly by resibufogenin. The mechanism of the effect on respiration was considered to be excitation of the respiratory center. Compared to previous reports, the excited effect of resibufogenin on respiration was not abolished by the administration of procaine. It was suggested that this effect was mediated through the central nervous system. Therefore, resibufogenin is a central respiratory stimulant.

4) *Vasopressor action*. Resibufogenin produces an increase in mean arterial blood pressure significantly in hemorrhaged animals. (Shimizu, 1916; Chan *et al.*, 1931; Iwatsuki *et al.*, 1965; Leigh and Caldwell, 1969; Xie *et al.*, 1975; 1977; 2000; 2001). This vasopressor effect of resibufogenin appeared to be predominantly due to its peripheral vasoconstrictor action, and at least in part due to increasing cardiotonic action and cardiac output, without any change in heart rate. The peak rise in arterial blood pressure was observed at about 3 min and lasted between 30 and 60 min (Okada *et al.*, 1960; Suga, 1973; Morishita *et al.*, 1992, Xie *et al.*, 1977; 2000; 2001).

5) *Antitumor activity*. Bufalin and the formations of venom induced apoptosis in human leukemia cells and inhibited ehrlich ascites tumor cells in mice. Also, bufalin has differentiation-inducing activity in several myeloid leukemia cell lines (Jing *et al.*, 1994; Yamada, *et al.*, 1998; Kawazoe, *et al.*, 1999a, 1999b).

6) *Anti-inflammatory effect*. Toad venom has a strong anti-inflammatory effect and decreases capilary permeability. This substance increases the activities of the cells of the reticuloendothelial system (Bensky and Gamble, 1993).

7) *Diuretic*. Almost 90 years ago, Abel and Macht (1912) observed that a small dose of bufagin caused vaso-dilatation in the kidney at a time of constant or higher blood pressure and vaso-constiction elsewhere in the body (Abel and Macht, 1912). Recent report showed that toad venom reduced the tubular reabsorption of Na^+ and Cl^- (Huang 1999).

THE ELECTROPHYSIOLOGICAL EFFECT OF RESIBUFOGENIN

Electrophysiological experiments were performed on dog, sheep, rabbit, guinea pig, and human heart tissue *in vitro,* using standard glass microelectrode techniques and *in vivo*, on the intact heart using monophasic action potential techniques (Xie and January, 1993). These experiments demonstrated that resibufogenin is very similar to digitalis (Xie et al, 1985a; 1985b; 1988a; 1988b; 1988c; 1993; 1994). The basic effects of resibufogenin on transmembrane and monophasic action potentials showed that there were progressive decreases in all parameters of action potential. The typical data are summarized in Table 2. Concisely, there are three major effects on the electrophysiological parameters. 1) Resibufogenin decreased the absolute value of the resting potential and the maximal diastolic potential. 2) Resibufogenin shortened the action potential duration both in membrane potential and in monophasic potential. 3) Resibufogenin decreased action potential amplitude both *in vitro* and *in vivo*. In addition, resibufogenin in different concentrations (0.15, 0.3, 0.6, 0.9, 1.4 mM) (Xie *et al.*, 1988b) had a concentration-dependent effect on electrophysiology. A comparison study between resibufogenin and acetylstrophanthidin was performed on sheep Purkinje fibers by microelectrode and extracellular electrogram techniques (Xie *et al.*, 1994). The results indicated that the electropharmacological characteristics of resibufogenin were similar to those of acetylstrophanthidin, including all parameters of action potentials, electrograms, and time course of the effect, etc. This strongly suggests that resibufogenin belongs to this family of digitalis-like drugs.

ADVERSE EFFECTS

For a long time that toad venom is a quite toxic compound of Chinese herbal medicine. Clinically, the signs and symptoms of toad venom poisoning

Table 2. Electrophysiological effects of resibufogenin (RBG) on transmembrane and monophasic action potentials in canine Purkinje fibers *in vitro* and in rabbit hearts *in vivo*

	In Vitro (Canine Purkinje fiber)		*In Vivo* (Rabbit beating heart)	
	Control	RBG (0.6 μM)	Control	RBG (0.3 mg/kg)
APA (mV)	118 ± 6.1	102 ± 8.8*	30 ± 6	17 ± 4**
APD50 (ms)	---	---	92+/-13	87+/-17*
APD75 (ms)	296 ± 62.1	254 ±36.6	---	---
APD90 (ms)	---	---	117 ± 15	109 ± 16**
V_{max} (V/s)	312 ± 38.8	200 ± 52.1*	---	---
RP (mV)	-88 ± 7.5	-74 ± 10.4*	---	---
HR (bpm)	---	---	280 ± 24	249 ± 38**
Fe (g)	---	---	23 ± 5	28 ± 6**

Values represent mean ± SD. Compared with controls: *$P < 0.05$; **$P < 0.01$. APA: Transmembrane action potential amplitude *in vitro* and monophasic action potential amplitude *in vivo*. $APD_{50,75,90}$: Action potential duration at 50, 75 and 90% of repolarizations both *in vitro* or *in vivo*. V_{max}: the maximum rate of rise of transmembrane action potential phase 0. HR: Heart rate. Fe: Contractile force.

may include local irritation with profuse salivation, perioral numbness, nausea, vomiting, diarrhea, blurred vision, hypotension, and abdominal pain. According to a previous report in England (Hitt and Ettinger, 1986), the ingestion or mouthing of some toads is well known by veterinarians to cause severe intoxication, often with fatal outcomes. Salivation, cyanosis, and convulsive seizures are cardinal clinical signs of bufotoxin ingestion in dogs. In severe poisoning, neurological effects are the most pronounced clinical feature. The cardiovascular toxicity of venom includes cardiac arrhythmias (ventricular fibrillation, tachycardia, bradycardia, second-degree atrioventricular block, irregular heart beat). CNS toxicity includes dizziness, and numbness of the extremities (Chern, 1991; Yei and Deng, 1993; Ko *et al.*, 1996; Huang, 1999).

Most seriously, there were some reports in the U.S. of toxicity of toad venom. Cardiac arrhythmias and other toxicities were induced by these Chinese medicines and the toad venom containing drugs (Hitt and Ettinger, 1986; Chern *et al.*, 1991; Kwan *et al.*, 1992; Brubacher *et al.*, 1995; Brubacher *et al.*, 1996; Barry *et al.*, 1996). One case of toad venom-induced digitalis toxicity by Yixin Wan, a toad venom containing drug was presented in the U.S. Kwan *et al.*, (1992) have suggested that this medication not only caused a digitalis-like effect, but also was responsible for digitalis toxicity in the patient. Another case reported a patient who consumed a bowl of toad soup, stewed with rice wine. The toxic effects of toad venom can be attributed to the glycosidic portion of the bufotoxin complex (Chern *et al.*, 1991).

An animal study (Brubacher *et al.*, 1999) showed that toad venom caused irritability, seizures and death. Following venom administration an apparent change in animal behavior was evident. For the first several minutes following injection, animals became more active and ran rapidly around the cage. Subsequently, the animals became less interactive, and would sit on their hindfeet with their fur puffed out and seemed to ignore other animals. Some animals would have convulsions and recover, but death was always preceded by covulsions. In the last stage of the experiment, 30 of 30 control mice had seizures and died within 3 h following venom administration. In both *in vivo* and *in vitro* experiments, resibufogenin induced delayed afterdepolarization and triggered arrhythmias (Xie *et al.*, 1985a; 1985b; 1988a; 1988b; 1994; 2000; 2001).

The LD_{50} of Chan su in mice was 41.0 mg/kg (i.v.), 96.6 mg/kg (s.c.), and 36.2 mg/kg (i.p.) (Bensky and Gamble, 1993). A recent report (Brubacher *et al.*, 1999) showed that the LD_{90} of Chan su was 49.4 mg/kg. The LD_{50} of resibufogenin was 16.61 mg/kg (i.p.) and 6.1 mg/kg (i.v.) (Xie *et al.*, 1975; 1977).

Presently, nonstandard therapies can be used for patients poisoned by toad venom. Because toxicity of toad venom is similar to digoxin, the antibodies used in the assays of digoxin react with toad venom (Fushimi *et al.*, 1990; Brubacher *et al.*, 1996;

Dasgupta, and Emerson, 1998; Biddle *et al.*, 2000; Oda *et al.*, 2001). The results showed that digibind can bind cardiac toxins, such as bufalin and cinobufotalin, *in vitro*, thus reducing free concentrations. Bagrov *et al.* (1993) have shown that antidigoxin antibodies not only bind to toad venom, but also that they block the ability of toad venom to inhibit the Na^+-K^+-ATPase. The use of digibind for the treatment of toad venom poisoning *in vivo* resulted in similar results (Brubacher *et al.*, 1999).

CONCLUSIONS

Toad venom is a valued traditional oriental medicine obtained from the skin venom gland of the toad and has been widely applied in China, Japan, Koran and other oriental countries for a long time. Toad venom contains multiple biologically active substances that have multiple pharmacological actions. Also, toad venom has certain toxicity and adverse effects both in animal experiments and clinically. However, at present, nonstandard therapies can be used for patients poisoned by toad venom.

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