Review Article
Angel of human health: current research updates in toad medicine

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Abstract: There are currently 34 genera and 410 species of toads in the world. The medicinal parts of toads mainly include their venom, skin, and clothing. The toad's venom and skin possess the same chemical components, mainly the toad venom lactone class, and their pharmacological effects primarily include the maintenance of strong heart, antitumor, antivirus, anti-infection, and analgesic effects. So far, the produces from the medicinal raw materials of the toad are widely used clinically around the world, especially in China, Japan, and South Korea. About 50 varieties of medicines are used in the clinical treatment of various complicated diseases in China, such as "Liushen pills" which was popular in the whole world. Toads are mainly used in treating malignant tumors (*e.g.*, liver cancer, gastric cancer, esophageal cancer, colon cancer, cervical cancer, among others), and some major diseases such as hepatitis B. Despite the therapeutic effects of toad-derived medicines on human health, there is insufficient research and development of toad-derived medicines by leading drug companies. In order to harness the beneficial effects of the resources of the toad species, it is the responsibility of global pharmaceutical researchers to develop and generate economically feasible toad-derived therapeutic products, while promoting maximum protection to the resources of the toad species.

Keywords: Toad, cancer, hepatitis B

Introduction

As a creature, the toad has made outstanding contributions to human health. There are 34 general and 410 species of toads all over the world that includes Australia as well as Madagascar [1-3]. Toads are probably the most common amphibians in China. Within the Bufonidae family, more than half of all species belong to the same genus, namely the toad. Bufo members of this genus are the most predominant species.

There are 2 general, 17 species or subspecies of toads in China [4]. The three main varieties are: 1) the *Bufo bufo gargarizans*, 2) the *Bufo raddei*, and 3) the *Bufo melanostictus*. Toads have a bloated shape, and their bodies are full of bumpy warts, hence they are known as *Laihama*, and are hailed as the world's ugliest animals. However, they have an indelible func-

tion in medical history, and have been recorded in China's *Zhang Zhongjing* (Classical Prescription), *Sun Simiao*'s "Valuable Prescriptions for Emergencies", *Hua Tuo*'s "Hua Tuo Reengineering", and *Li Shizhen*'s "Compendium of Materia Medica". Today, the toad continues to be essential for the health of mankind. Toads have been recorded as an invaluable source of medicine for humans in ancient and modern Chinese medicine, as well as in modern scientific research.

Modern research in toad medicines

At present, the components of toads that can be directly used as medicine are mainly toad venom, toad skin, and toad clothing. The mainly composition were approximately 103 toad venom ligand compounds and 12 indole alkaloids as chemical constituents in toad [5]. The main composition of dry toad, toad skin and
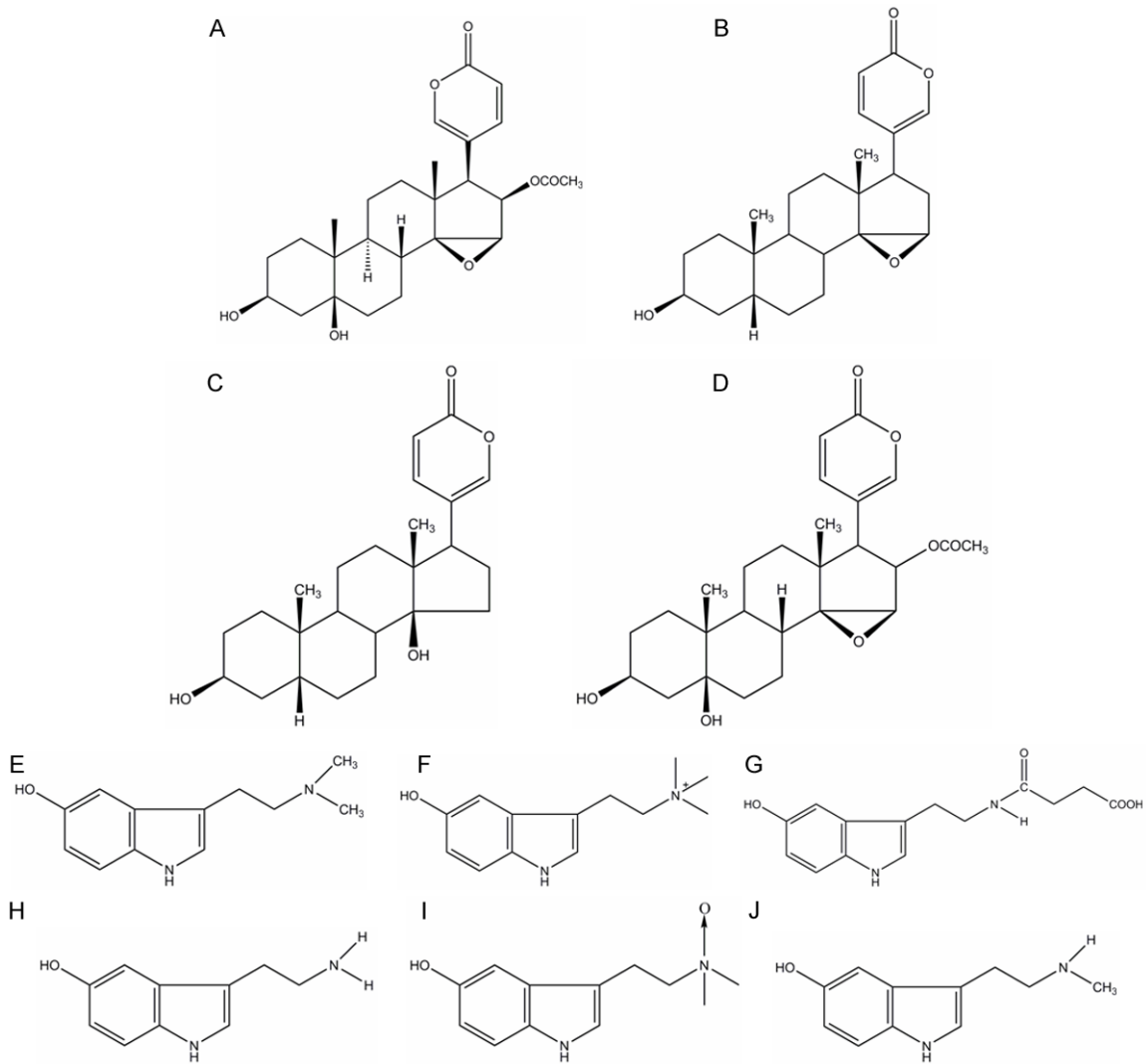
Figure 1. Structure of main chemical ingredients in the toad venom. A. Cinobufagin (CB). B. Resibufogenin (RBG). C. Bufalin (BL). D. Cinobufotalin (CBTL). E. Bufotenine (BT). F. Bufotenidine (BTD). G. Bufobutanoicacid; H. Serotonin. I. Bufotenine N-oxide. J. N-methyl serotonin.
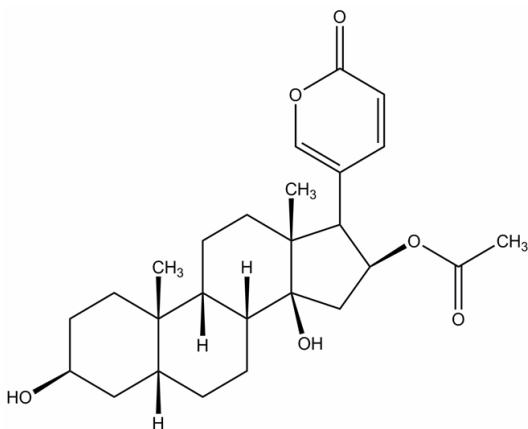

Figure 2. Chemical construction of Bufotaline.

toad clothing compared to toad venom is basically the same; however, the difference in the concentration of the components is large. Thus, the differences in pharmacological functions and clinical applications of the varied toad medicines are very distinct.

The chemical composition of toad venom

Toad venom is the most complex composition of toads. The difference in chemical composition varies greatly based on the variety of the toad venom, area of production, differences in the methods of collecting and processing. Toad venom has both fat soluble and water-soluble chemical components, based on the active
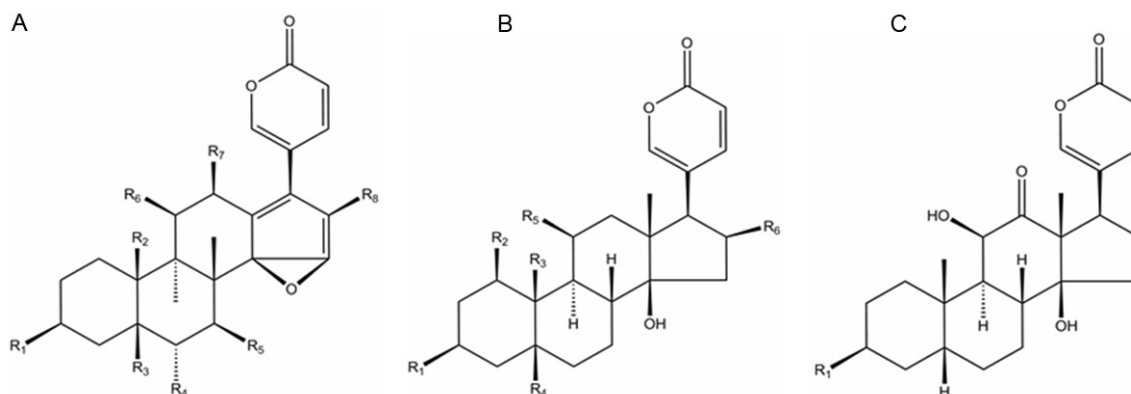
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Figure 3. Toad alkadiene acid lactone compounds. A-C. Showed kinds of the mother structures.
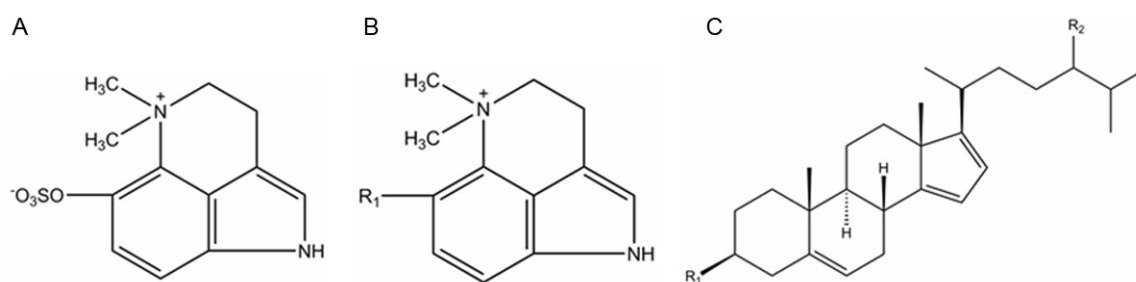

Figure 4. The class indole alkaloids. A. Toad thiamethoxam nowadays. B. Sterol compounds. C. Chemical structure of the mother structures.

ingredient that influences the dissolving properties. In order to determine the composition of the chemical structure of the fat-soluble class in toad venom, it has been observed that cinobufagin ($C_{26}H_{34}O_6$, CBG, **Figure 1A**), resibufogenin ($C_{24}H_{32}O_4$, RBG, **Figure 1B**), and bufalin ($C_{24}H_{34}O_4$, BL, **Figure 1C**). These three chemicals account for about 10% of the dry weight (CBG or RBG content in a small number of samples of toad venom can be as high as 7 to 8%) [6]. Cinobufotalin ($C_{24}H_{34}O_6$, CBT, **Figure 1D**), BL, CBG, and RBG content in the range of 0.05-5.12% (w/w), are derived from *Bufo bufo gargarizans* of toad venom, and the concentration of of RBG, CBG, BL, CBT are in the range of 0.94-2.15%, 1.19-3.03%, 0.46-1.07% and 0.29-0.87%, respectively. The concentrations in toad venom from *Bufo melanostictus* are in the range of 0.12-3.69%, 0.19-5.12%, 0.08-1.71% and 0.05-1.14%, respectively [7]. Water-soluble ingredients include bufotoxins such as the toad diene class, the cardiac glycosides ene toad venom, the indole alkaloids (**Figure 1E-J**), the alcohols and polysaccharides, the amino acids, the peptides, adrenaline, toad venom tryptamine like serotonin, toad venom tryptamine, toad venom quaternary ammonium, toads and ning, and dehydrogenation toad venom tryptamine, toad venom tryptamine hydrobromide, and others [8].

Toad toxins and toad color amine content have the strongest pharmacological effects in toad venom. Bufotalin ($C_{26}H_{36}O_6$, BTL, and **Figure 2**) is one of the most important active compounds. Yang et al [9] separated toad venom spirit, 3-butyl-2-acyl arginine acetate, from the toad skin. Shimada, et al [10] extracted spirit-3-symplectic-2-acyl-L-histidine, 1-methyl-3-methyl histidine, histidine ester and five other toad toxins from the toad venom and the skin of toads, in Taiwan and *Bufo melanostictus*.

Cardiac steroid compounds, can be divided into free-type and combination-type compounds. The free-type compounds are called bufogenin, such as BL, RBG, and others. Combination-type compounds are called bufotoxin, such as fat toad venom ligands, 3-sulfuric acid esters. These compounds are toad diene hydroxyl acid lactone compounds (**Figure 3**) [11].
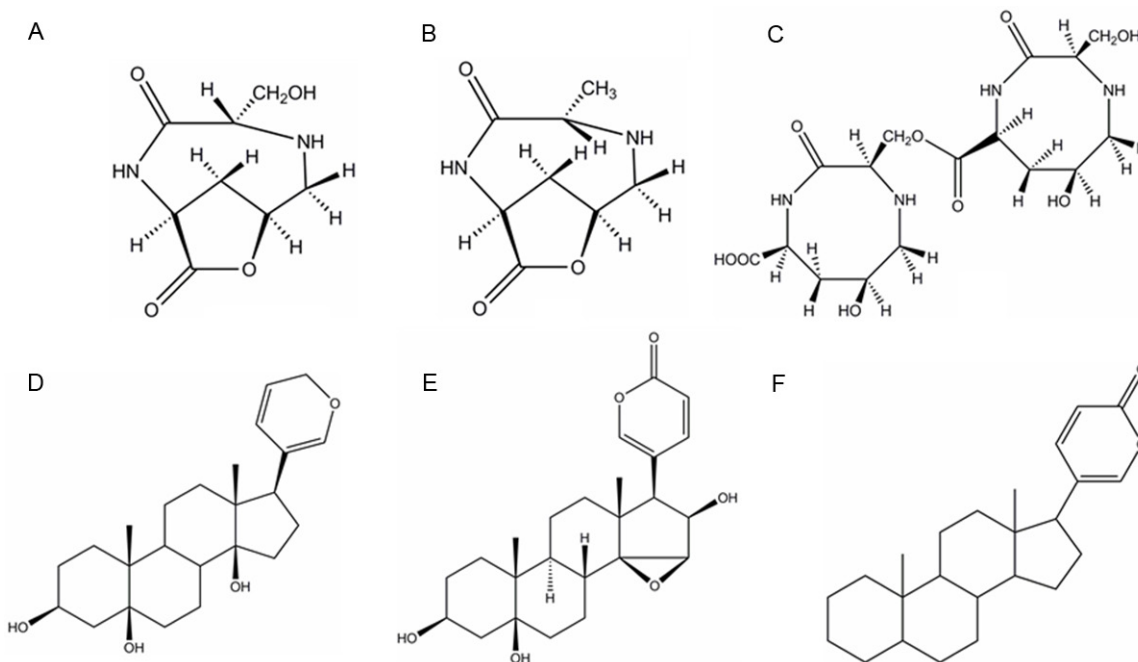
Figure 5. Toad ring amide compounds structures. A. Bufogargarizaine B. B. Bufogargarizaine D. C. Bufogargarizaine C. D. Telocinobufagin. E. Desacetylcinobufotalin. F. Hellebrigenin.

Toad venom lubricious amine compounds belong to the class of indole alkaloids. Li Weixi [12] isolated toad thiamethoxam from the dry toad (**Figure 4A**). The chemical composition content of toad thiamethoxam is high in the dry toad and is low in the toad venom. The dry toad also contains cholesterol, beta sitosterol, and palmitic acid cholesterol ester (**Figure 4B**, **4C**).

Such compounds are divided into compounds containing lactone structure, such as the toad ring amide B ($C_8H_{12}N_2O_4$, *bufo gargarizans*-B, BGZ-B, **Figure 5A**) [13], the toad ring amide D ($C_8H_{12}N_2O_3$, *bufo gargarizaine*-D, BGZ-D, **Figure 5B**) [14], and exclude lactone structure compounds, such as toad ring amide C ($C_{16}H_{26}N_4O_9$, *bufo gargarizans*-C, BGZ-C, **Figure 5C**) [13].

It has been reported that the water-soluble components that have been separated from the dry toad, contain ingredients like dipeptide, pyrimidine, and adenosine [14]. In addition, they also contain photopigments [15] and all kinds of inorganic elements [16].

The chemical composition of toad skin

The moisture content of toad skin was 10.99% on an average, the total ash content on an average was 13.21%, the acid insoluble ash content was 4.58% on an average, the CB and RBG contents were 0.13% and 0.03%, respectively [17]. Yan Ziping [18] performed 10 times the amount of 80% ethanol reflux extraction of toad skin, the average concentration of CBG and RBG, in the preparation of the extract, was 0.20% and 0.11%, respectively. There is a major impact on the toad skin when using different extraction methods to extract ingredients like toad venom lactone. Cao Xutao et al [19] were able to extract RBG, CBG, BL, telocinobufagin ($C_{24}H_{34}O_5$, TCBG, **Figure 5D**), BTL, desacetylcinobufotalin ($C_{24}H_{34}O_6$, DTCBT, **Figure 5E**), hellebrigenin (HBG), arenobufagin (AB), gamabufotalin (GBTL), 11β-hydroxylresibufogenin (11β-HRB), CBT, and other 11 kinds of bufogenin components from the toad skin. Zhao Dazhou et al [20] did a comparative study on toad diene lactone compounds and their concentration in the toad venom and the toad skin, and found that both contain the same diene lactone composition that mainly includes four indole alkaloids. The concentration of dehydrogenation toad tryptamine is higher in the toad skin, while it is low in toad venom. Sterols in the toad venom mainly consist of sitosterol, while in toad skin; the sterols are mainly cholesterol and cholesteryl palmitate. The skins of the Taiwan toad (*Bufo vulgaris for-*

mosus Boulenger), green toad (*B. viridis* Laur?) from Japan, Bangkok toad (*B. bankorensis* Borbour) from Taiwan and *Bufo Bufo gargarizans* (*B. Bufo gargarizan* Cantor) from China were analyzed and found to possess succinic acid, adipic acid and pimelic acid instead of symplectic 2 acyl arginine ester compounds, and sulfuric acid ester compounds. The skins of the toad (*B. americanus*) from North America and *Bufo melanostictus* (*B. melanostictus* Schneider) from Taiwan were analyzed and shown to contain I-histidine, L-1-methyl histidine, and L-part 3 instead of arginine-methyl histidine-bufatoxin compounds [21]. Therefore, the chemical composition and the concentrations in toad skin from different regions in the world display a large difference. Xu Naiyu et al [16] analyzed and determined the chemical elements in the toad skin of *Bufo gargarizans* from China, and showed that the toad skin contains calcium, magnesium, sodium, manganese, iron, zinc, copper, phosphorus, silicon, and the silver elements. The calcium content is the highest, followed by iron and magnesium.

Chemical composition of toad clothing

Chinese scholars [16] first analyzed the chemical composition of toad clothing from *Bufo gargarizans* and identified eight compounds: 1) hexadecanoic acid cholesterol ester, 2) cholesterol, 3) 5-alpha, 8-alpha-epidioxycholesta-6-en-3 beta-ol, 4) cholesteric-5-ene-3 beta,7 beta- diol, 5) cholesteric-7-ene-3 beta, 5 alpha, 6 beta-three glycol, 6) 3-18 alkoxy-1, 2-propylene glycol, 7) delta^{4,5}(*E*), delta^{9,10}(*Z*)-sheath amino alcohol (fifteen carbonic acid amides), and 8) toad thiamethoxam. The compounds (3), (5)-(7) were obtained from *Bufo gargarizans* and the pseudopelade at the first time. In addition, other reports have documented that toad clothing contains RBG in the range of 31.3-80 μg/g [22-24].

Pharmacological research

Cardio tonic effect

The effect of RBG, in toad venom, on cardiac function is stronger, followed by BL and CBG. Studies have shown that the impact of CBG on anesthesia, blood pressure, and normal cardiac function is not obvious; however, there is a positive effect on the artificial blood loss-induced hypotension of the drug. There is an increase in cardiac output and an increase in arterial pressure, but the impact on the heart rate is not obvious [25]. The inhibitory effect of the toad venom on Na$^+$-K$^+$ ATPase in red blood cells is very strong, and leads to an increased myocardial intracellular Na$^+$ concentration. The Ca^{2+} levels in the myocardial cells get excited and reinforce a myocardial contraction [26]. Toad venom can prolong the fibrinogen coagulation time; its anticoagulant effect is similar to urokinase, by making the fibrin dissolve activated enzymes that increase the coronary artery perfusion flow and the myocardial nutritional blood flow to improve microcirculation, and thus increases the myocardial oxygen supply. In addition, the toad venom can cause peripheral vascular contraction thereby mimicking the effect of adrenaline. Xie Jingtian [27] confirmed RBG can decrease membrane reaction in Pu Ken wild fiber of dog and sheep, slow conduction and generate excitement anti-arrhythmic drugs.

Anti-tumor effect

Antitumor activity is one of the most important pharmacological effects of toad venom. The IC$_{50}$ for toad steroid alkene compounds on tumor cells is in the range of 1 to 10 nM. The antitumor mechanism involves inducing tumor cell apoptosis, promoting tumor cell differentiation, increasing immunity, and inhibiting proliferation and angiogenesis of tumor endothelial cells [28]. Kamano [29] tested 80 types of natural toad steroid materials and its chemical derivatives on liver cancer cells. The *in vitro* inhibitory effect of PLC/PRF/5 showed that the C-17 alpha-pyrone ring, 3 beta-OH, 14 beta-OH (or 14 beta, 15 beta-ternary ring oxygen), C/D ring cis-fused groups, and other similar structures are necessary for the antitumor activity. When 19-methyl oxidized into aldehyde group the activity was enhanced, and when the 14-is a beta-OH of toad steroid ene role the activity was stronger than 14 beta and 15 beta with ternary oxygen ring structure, and HBG, BL in all subjects the strongest active compounds (IC$_{50}$ were $1.6×10^{-4}$ μg/mL and $2.8×10^{-4}$ μg/mL). All the toad steroids contain 14 beta and 15 beta ternary ring oxygen compounds, where the activities are the strongest (IC$_{50}$ = $7.4×10^{-4}$ μg/mL). In such compounds 19-oxidation of aldehyde group activity was enhanced, while the

hydroxymethyl activity was weakened; the activity of 16-bit acetoxyl group was significantly enhanced, while introducing the hydroxyl or other ester base was reduced [30]. Chen Xiaoyi et al [31] found that BL inhibits the growth of the poorly differentiated gastric cancer, MGC-803, and the IC_{50} is about 0.1 μmoL/L. Yin Peihao et al [32] observed that BL significantly inhibits BxPC-3 pancreatic cancer cell growth. There is a positive correlation between inhibition, drug concentration, and the time of action. Su Yonghua [33] showed that the different concentrations of BL damage the human liver cell membrane. BL, rather than CBG and RBG, has a pronounced inhibitory effect on SMMC-7721 and BEL-7402, and that the same effect on growth inhibition was lower than that of mitomycin. The team of Qu Junle [34] found that BL significantly inhibits the growth of ASTC-a-1 cancer cells from human lung adenocarcinoma in the form of concentration dependence. Jiang [35] verified that BL has an obvious pharmacological activity on the human non-small cell lung cancer cell line A549, and this inhibition activity was positively correlated with the time of administration and the dose of the drug. The IC_{50} of BTL on human Hela cells, PLC/PRF/5 cells, human nasopharyngeal carcinoma Kb cells, and human granulocyte leukemia HL60 cells are 0.11 μM [36], 3.4×10^{-4} μg/mL [29], 0.19 μg/mL/mL [37], and 0.01 μg/mL [38], respectively. Su [38] found that IC_{50} for BTL-treated human hepatoma Hep3B, human colorectal cancer HT-29, and human breast cancer MCF7 cells, after 6 days of differentiation, were 0.24 ± 0.02 mM, 0.234 ± 0.03 μM, and 0.15 ± 0.02 μM, respectively. The above results show that the BTL antitumor effect is not only significant, but BTL has a wide antitumor spectrum. Tian [39] detected the antineoplastic activity of 10 kinds of bufadienolide compositions on HepG2 and A549 cells, and found that the IC_{50} of BL were 0.6 ± 0.0 μM and 0.6 ± 0.1 μM, and the IC_{50} of BTL were 0.5 ± 0.1 μM and 0.9 ± 0.2 μM, respectively, compared with that of adriamycin (0.3 ± 0.1 μM and 0.1 ± 0.0 μM) that showed no significant difference. Belgian scholar Moreno [40] performed a comparative study of the antitumor effects of 27 kinds of bufadienolides in 6 strains of anthropogenic tumor cells, Hs683, MCF-7, PC-3, A549, U373, SKMEL-28, and tumor cells of 2 strains of the rat, CT26 and WT B16F10. The confirmed average IC_{50} of the gamabufotalin

rhamnoside, bufotalin, hellebrin and argentinogenin were low, ie, 50 ± 11 nM, and the IC_{50} of the gamabufotalin rhamnoside on 6 kinds of anthropogenic tumor cells were low, ie, 3.0 ± 0.0 nM. The antitumor mechanism of bufadienolides may either involve inhibition of the Na^+-K^+ ATPase activity of tumor cells or ClC-$3Cl^-$ is catalyzed to start inhibiting the P13K/Akt/mTOR signaling pathways, which have an antitumor role [41].

Induction of tumor cell differentiation

BL is the main component in inducing tumor cell differentiation [42]. The main mechanism is to inhibit topoisomerase II and the activities of protein kinase A and protein kinase C. By lowering WT1 gene expression, BL induces differentiation of K562 cells. When BL is in the range of 0.01 moL/L-0.026 moL/L, there is only cell differentiation, and when BL is in the range of 0.026 μmoL/L-0.05 μmoL/L, WT1 protein and mRNA levels will be lower. It has been shown that decreased WTI gene expression can promote differentiation and apoptosis of K562 cells [43]. Research by Takai [44, 45] showed that endometrial cancer cells (HHUA, HEC-1B), ovarian cancer (SKOV3, OMC-3), placental villi cancer cells BeWo, and gynecological malignant tumor cells are extremely sensitive to BL, and the drug mainly inhibits tumor cell differentiation in the G0/G1 phase.

Induction of apoptosis of tumor cells

Huang [46] found that BL, at a concentration of 0.01 μmoL/L, inhibits the growth of human leukemia cells HL60 and apoptosis occurs after 24 h in the cells. Bone sarcoma cells, U2OS, treated with BL for 48 h, display condensed chromatin and have the typical apoptotic body that increases with the increase in the concentration of BL. This indicates the cytotoxicity of BL and its effect on promoting apoptosis [47]. BL has an effect on target-induced tumor cell apoptosis, for example, BL can increase the expression of the Bax protein and lower the Bcl-2 protein levels, to induce the apoptosis of tumor cells (eg, U2OS, HL60, HepG2, A549) [48]. Sun [49] studied and found that when BL acts on human lung adenocarcinoma ASTC-a-1 cells, it can produce a large number of reactive oxygen species, strengthen caspase 3 activity at the same time, induce the transfer of the Bax apoptosis protein from the cytoplasm to the

mitochondria, thereby inducing apoptosis in ASTC-a-1. Kurosawa [50] found that specific inhibitors of protein kinase c (protein kinaze c, PKC) can significantly decrease the leukemia expression of BL-inducing IL-1 beta gene of the THP-1 human mononuclear cells, and thus explain that BL may induce differentiation and apoptosis of the tumor cells through the PKC pathway. Recent studies suggest that the membrane Na^+-K^+ ATPase is the main target of BL and has an anti-tumor effect, through specific inhibition of Na^+-K^+ ATPases, and by launching a series of activated downstream proteins related to apoptosis signaling pathways, thus, eventually leading to apoptosis [51, 52].

Autophagy induced by tumor cells

Our researches as well as previous reports [53] have confirmed that BL can significantly cause apoptosis and autophagy of human hepatocellular carcinoma HepG2 cells [54]. It has also been confirmed that BL causes cell death in human colon cancer cell lines, HT-29 and Caco-2, by inducing autophagy and not by inducing apoptosis. The mechanism of autophagy involves BL to induce cancer cells to produce a lot of reactive oxygen species (ROS). In turn, ROS activates the JNK pathway-raised autophagy related genes *ATG5* and *BECN1* expression and initiates the autophagy of tumor cells.

Anti-angiogenesis

Studies by Lee [55] confirm that 5, 10 and 20 nmol/L of BL have significant inhibitory effects on angiogenesis, and the inhibition rates are 45.3%, 62.8%, and 75.6%, respectively. Wang Nayao [56] found that *Huachansu* injection contained the BL component in the chicken embryo allantois membrane and had an inhibitory effect on angiogenesis.

The analgesic effect of anesthesia

Zhang Wei [57] confirmed that toad venom has an analgesic action with 6 kinds of fat-soluble components, of which 1.3 mg/kg RBG and 1.3 mg/kg CBG display the most significant analgesic effects. The analgesic effect of 0.8 mg/kg BL is relatively stable, and the analgesic effect of the South American toad toxins, BL and BTL, are weaker than the CBG. The local anesthesia effect of BL is 30 to 60 times more than cocaine, 300 times more than procaine, and

the anesthesia time is long. There are no central poisoning symptoms and local stimulation, and the mechanism may be associated with the slow release of acetylcholine of muscle cells [58].

Other activity

Toad venom exerts rapid and significant antibacterial effects on *Staphylococcus aureus* and alpha hemolytic *Streptococcus*. Toad venom also displays therapeutic effects on purulent disease induced by some antibiotics, but also has the capacity to increase blood capillary permeability during inhibition, reduces medicinal overflow, and is beneficial in eliminating swelling, and so on. CBG activates the mice abdominal cavity macrophages, improves phagocytosis, kills bacteria and inhibits the growth of bacteria [59]. Studies confirm that the toad clothing extract can inhibit Na^+-K^+ ATPase activity [60], reduce blood sugar [61], inhibit HIV-1 [62], and inhibit mice S_{180} sarcoma, H_{22} liver cancer, Lewis lung cancer, liver ascites, and HCA growth pharmacological effects, among others [63].

Toxicity

Toad venom

The oral LD_{50} of toad venom is 0.36 mg/kg, and the common adult clinical oral dose is 3-5 mg/day (the maximum dose cannot exceed 135 mg/day) [64]. He Shilin [65] confirmed the LD_{50} of toad venom extracts by different fabrication processes. Intravenous injection of mice produced a rough extract of 0.04 g/kg of toad venom, an alcohol extract of 0.21 g/kg, and a water extract of 0.9 g/kg of toad venom. Toad venom, produced by intravenous injection, of 75% ethanol extract in mice for 14 days showed an LD_{50} of 0.60 g/kg [66]. The intravenous LD_{50} of toad venom was 41 mg/kg, LD_{50} of toad venom by subcutaneous injection was 96.60 mg/kg, and that by intraperitoneal injection was 36.24 mg/kg [67].

Toad skin

A research of Jin Qiquan [68] shown that a mixture of toad skin water and fat displayed an intravenous LD_{50} of 3.81 ± 0.22 mg/kg in mice, and an intraperitoneal injection showed an LD_{50} of 26.27 ± 0.30 mg/kg. It was observed that the original toxic drug ingredients are

mainly toad toxins and ester soluble constituents.

Lactone class ingredients of toad venom

The LD_{50} by intraperitoneal injection of BL and CBG were 2.22 mg/kg and 4.38 mg/kg, respectively. The LD_{50} of toad aglucone by rapid intravenous injection was 4.25 mg/kg, by slow intravenous injection was 15 mg/kg, the LD_{50} by intraperitoneal injection was 14 mg/kg, the LD_{50} by subcutaneous injection was 124.50 mg/kg, and orally, the LD_{50} was 64 mg/kg. The LD_{50} of toad turney intravenous injection was 1.30 mg/kg. The intravenous LD_{50} of BTL in dogs is 0.36 mg/kg, with an oral minimal lethal dose of 0.98 mg/kg. The LD_{50} of BL mice tail intravenous injection was 2.26 mg/kg [69]. The LD_{50} of BTL tail intravenous injection in mice was 4.13 mg/kg [70].

Secretio bufonis injection

The LD_{50} of rat peritoneal injection was 102.65 mg/kg [71]. The main adverse reactions of the toad venom injection in clinical use are phlebitis, rare allergic reactions and arrhythmia, but most of the symptoms can be controlled by regulating the dripping speed, preventing leakage, and careful nursing that are effective to prevent these side reactions. Other general side effects of oral preparations are lighter and can be effectively prevented through a control dose [72].

Cinobufacini injection

Our study confirmed that the LD_{50} of the intravenous Cinobufacini injection in the rat was 312.95 mg/kg. Clinical side effects mainly are high-risk vascular irritation reaction (about 70.63%), followed by drug fever (10.71%), and allergic reactions (9.13%) [73].

To sum up, the toad lactone class is the main medicinal ingredient. The toad lactone is a lip soluble constituent, where the most important toxic target organ is the heart. The intensity of toxicity decreased in the order of AB>BL>TCBG>HBG>GBTL>CBT>BTL>RBG>DTCBT [74].

Drug research

Toad venom as raw materials for drugs

In China, toad venom has been made into a variety of drugs, such as injections, oral liquids, film agents, pills, the transdermal drug delivery system, among others, and is mainly used for clinical treatment of multiple premature beats, increase in eosinophils, leukemia, skin cancer, neurodermatitis, tuberculous fistula, toothache, and local anesthesia [75, 76]. In recent years, some scholars have been conducting research in new dosage forms, mainly liposomes, microspheres, beta entrapped cyclodextrin complex, albumin nanoparticles, aerosols, Papua agents, the microemulsion combine, and others [77, 78]. For example, injections made by Chinese *Anhui Keyuan* pharmaceutical group Co. Ltd. (approval number: Z34020604) and the Chinese *Jiangsu Ange* pharmaceutical Co. Ltd. (approval number: Z32020694), with main ingredients called indole alkaloid derivatives, indoles, total alkaloid in serotonin ($C_{10}H_{12}ON_2$) per milliliter meter >18 μg, have effects in heat-clearing and detoxification; *Liushen* pills (approval number: Z32020481), the compound toad venom mastic (approval number: Z20063321), *Tianchan* capsule (approval number: Z20020056), the toad venom *Jiuxin* pill (approval number: Z22022831), *Xinli* pill (approval number: Z44021844), Japan's approval and production of KYUSHIN (approval number: C310621) these medicines are widely used in kinds of cancer (digestive, respire atory, urinary and reproductive system, skin and head and neck cancer patients), a variety of diseases such as asthma, acute and chronic cardiac failure.

Toad skin as raw materials for drugs

Toad skin also has been made in kinds of medical preparations. Such as Cinobufacini injection which contains ingredients like toad venom ligands, with the concentration of the ingredients in the range of 4.62 to 5.80 mg/L [79], and shows detoxification, detumescence effects, pain relief, antiviral effects, promotes bone marrow proliferation, and enhances immunity effect, among other functions. The injection can be used in the clinical treatment of advanced tumors and chronic hepatitis B [80-82]. Its antitumor mechanisms include the drug-regulated immune function [83, 84], inhibition of tumor cell proliferation [85], induction of tumor cell apoptosis [86] and differentiation [87], inhibition of tumor angiogenesis [88], and reversal of multi-drug resistance [47]. The main representative products as Cinobufacini tablets (Z34020272), Cinobufacini oral liquid

(Z34020644), Cinobufacini capsule (Z2009-0944), *Antike* capsule (Z10960071), contains indole alkaloids, reducing sugars, amino acids, and toad toxins such as the toad tryptamine [89], widely used in detoxification, tumescence, pain relief, and the treatment of medium and advanced cancer, chronic hepatitis B, among other diseases.

Treatment of malignant tumors in clinical application

Cao Jie [90] confirmed that the toad venom injection in combination with the chemical therapy treatment of 60 cases of late malignant tumors, can significantly improve the body's immune function, improve the quality of life of patients, significantly increase white blood cells, and reduce the toxic reaction caused by chemotherapy. Zhao Jianqing [91] performed a comparative study of toad venom injection with chemotherapy and chemotherapy alone to analyze the effect on the treatment of advanced non-small cell lung cancer, in terms of toxicity and immune function. Radiotherapy with toad venom injection of 31 cases of patients with advanced esophageal cancer [92] and chemotherapy with toad venom injection of 34 cases of non-small cell lung cancer [93] confirmed that toad venom injection can significantly enhance the curative effect, relieve local pain, reduce fever, increase the appetite, improve the immune function and mental health, and other qualities of life. Huang Zhifen's [94] clinical observation confirmed that chemotherapy with toad venom injection in the treatment of advanced gastric cancer can act synergistically. Qiu Bingli [95] treated 60 cases of advanced malignant tumors with the Cinobufacini injection (34 cases of lung cancer, 16 cases of digestive tract tumor, 9 cases of primary liver cancer and I case of neurogenic tumor), and the total effective rate (PR+SD) was 68.33%. Wang Changjun [96] reports two methods, percutaneous left subclavian artery perfusion Cinobufacini injection and hepatic artery chemoembolization (HAC), to treat all the 30 patients with inoperable advanced liver cancer. The total effective rate of the Cinobufacini group was 70%, and significantly improved liver function and the prevention and treatment of liver fibrosis. The total effective rate of the HAC group was 76.7%. Ma Jinli [97] studied 109 patients with NSCLC (gemcitabine + cisplatin + Cinobufacini injection) and a control group of 108 cases (gemcitabine + cisplatin). The effectiveness of treatment group and control group were 55.96% and 37.96%, respectively. Leukopenia, abnormal renal function, and gastrointestinal reaction in the treatment group were significantly lower than the control group. Liu Xiaohong [98] reported that 42 cases of advanced primary liver cancer patients (HAC + Cinobufacini injection) compared with and a B group (pure HAC) with conventional treatment. The results showed the total effective rate of the A group was 83.3%, which is significantly higher than 57.1% of group B (P<0.01). The T cell subgroup number and NK cell activity of group A is significantly higher than group B. Cinobufacini injection and radiation combined treatment can obviously reduce the adverse reactions of tumor radiotherapy, improve the patients' tolerance.

Zhang et al [99] confirmed that Cinobufacini injection static drops companied with gamma knife treatment made side effects of radiotherapy and pain obviously lower than the control group without Cinobufacini injection static drops, and 1-year survival rate of the joint group was 73.33% higher than control group of 43.33% (P<0.01). Toad venom "*Jiening*'s" electuary treatment of advanced carcinoma of severe pain has the characteristics of quick effect, reduced time with pain, and less adverse reactions. The joint use of 20 tablets of morphine can increase the analgesic action [100]. The total effective rate of complex toad venom powder treatment of cancer pain was 93.3%, and the average duration of pain relief was 16.5 h. The improvement in the quality of life and the stability factor is 90% [101]. Liu et al [102] reported that the toad electuary was mainly used to treat various kinds of cancerous pain in 332 cases. The total effective rate was 92.65%. With *Chanwu* Cataplasm (the prescription is mainly composed of toad venom, radix aconiti, among others) treatment of 120 cases of patients with advanced lung cancer pain, 45 cases were greatly improved (37.5%), 60 cases were markedly improved (50%), 15 cases were invalid (12.50%), and 120 cases of patients had a pain duration of an average of 11.5 h [103]. Gong Zipeng et al [104, 105] observed that the analgesic effect of the Cinobufacini injection is mainly mediated by peripheral opioid receptors, and has nothing to do with the central opioid receptor. The analgesic action

may not produce similar effects as the central opioid receptor in blocking drug addiction and the withdrawal syndrome caused by side effects, but whether it is addiction needs to be further analyzed.

The toad venom injection, 10-20 ml + 5% glucose liquid, in a 500 ml intravenous drip was used to treat 50 patients of suspected influenza a (H_1N_1). The significant efficiency reached 90%. The comprehensive curative effect is superior to the Antondine and the radix bupleuri injection. Researchers [106] have observed this curative effect may be associated with the toad venom injection and displays antiviral and antibacterial effects, enhances immunity, relieves cough, and eliminates phlegm.

Cinobufacini injection suppresses HBV replication. The total effective rate was 55%, which was significantly higher than the control group (20%). The significant difference was found in the two groups (p<0.05) [107]. Pan Yiren [108] conducted the treatment of 50 cases of abnormal alpha-fetoprotein (AFP) in patients with chronic hepatitis B. one group was treated with 30 ml Ganlixin injection + 20 ml Cinobufacini injection and the control group with 30 ml Ganlixin injection. Results from the treatment group AFP showed a rapid decrease (including 18 cases dropped to normal, rate of 62.4%) compared with the control group (P<0.005). The normal control group showed no significant AFP, and the percentage is only 22.7%. In one year liver cancer was detected in only 2 cases (9.1%). Liu Hui [109] reported that HBeAg negative conversion ratios for the treatment group (add Cinobufacini injection) at the end of the treatment, or treatment after six months, were 62.90% (22/35) and 65.70% (23/35), respectively, while those of the control group were 37.90% (11/29) and 41.40% (12/29), respectively. HBV-DNA negative conversion ratios in the treatment group at the end of the treatment and 6 months after the treatment were 65.70% (23/35) and 68.60% (24/35), respectively while the control group was 41.40% (12/29) and 41.40% (12/29), respectively. This suggests that Cinobufacini injection can obviously improve the Ara-Amp negative conversion ratio of HbeAg and HBV-DNA, enhance the body's immune function, and improve the antiviral effect.

Epilogue

To sum up, toad, as an important member in the biological world, not only maintains the biological chain of balance and evolution, but also plays an important role in the protection of human health. Toads and humans live together on earth. Human beings should rationally protect the toad species in the earth's biosphere and promote the breeding of toads. Therefore, there should be more scientific and economic value for toad medicines that will guarantee the prosperity of the biosphere on earth, and hence, safeguard the health of humans.

Disclosure of conflict of interest

None.

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References

[1] Fei L, Ye CY and Jiang JP. A new bufonid genus parapelophryne from China (Amphibia, Anura). Acta Zootaxonomica Sinica 2003; 28: 762-766.

[2] Glaw F, Kohler J, Hofrichter R and Hofrichter A. Systematik der Amphibien: Liste der rezenten Familien, Gattungen und Arten. In: Hofrichter R, editors. Amphibien. Augsburg: Natur buch Verlag; 1998. pp. 252-258.

[3] Metts BS, Buhlmann KA, Tuberville TD, Scott DE and Hopkins WA. Maternal transfer of contaminants and reduced reproductive success of southern toads (Bufo [Anaxyrus] terrestris) exposed to coal combustion waste. Environ Sci Technol 2013; 47: 2846-2853.

[4] Wang GX. The toad, though ugly and have good fortune. Guide Sci-tech Mag 2013; 5: 37.

[5] Wu XY, Gao HM and Wang ZM. Advances on Chemical Constituents in Crude Medicines from Toads. China J Exper Trad Med Form 2010; 16: 207-214, 220.

[6] Ma XC, Zhang BJ, Xin XL, Huang SS, Deng S, Zhang HL, Shu XH, Diao YP and Cui J. Simultaneous quantification of seven major bufadienolides in three traditional Chinese medicinal preparations of chansu by HPLC-DAD. Nat Prod Commun 2009; 4: 179-184.

[7] Wong SK, Su XL, Liu CM and Lin RC. Study on quality control method for Venenum Bufonis. Chin J Pharm Anal 2011; 31: 1027-1030.

[8] Wang LM and Fan TT. Venenum bufonis. Med Infor 2013; 547-548.

[9] Yang LH, Zhang HZ, Zhang B, Chen F, Lai ZH, Xu LF and Jin XQ. [Studies on the chemical constituents from the skin of Bufo bufo gargarizans Cantor]. Yao Xue Xue Bao 1992; 27: 679-683.

[10] Shimada K and Nambara T. Isolation and characterization of a new type of bufotoxin from the skin of Bufo americanus. Chem Pharm Bull (Tokyo) 1980; 28: 1559-1562.

[11] Zeng Y, Zhang AJ and Wen X. The research progress of dry toad skin. China Med and Pharm 2011; 1: 29-31.

[12] Li WX, Li Q, Zhang XQ, Ye WC and Yao XS. The study of the Chinese big toad skin chemistry. Chin Trad and Herb Drug 2007; 38: 183-185.

[13] Dai LP, Gao HM, Wang ZM and Wang WH. [Isolation and structure identification of chemical constituents from the skin of Bufo bufo gargarizans]. Yao Xue Xue Bao 2007; 42: 858-861.

[14] Cao XT, Wang D and Wang N. Water-soluble constitutions from the skin of bufo bufo gargarizans cantor. Chin J Natur Med 2009; 7: 181-183.

[15] Yang LH, Jin XQ and Zhang W. Studies on the Chemical Constituents from the Skin of Bufo bufo gargarizans Cantor. J Shenyang Pharm Univ 2000; 17: 292-295.

[16] Xu NY and Gu ZL. Study on inorganic elements in Corium Bufo bufo garagarizans Cantor. Chin Trad Patent Med 2003; 25: 748.

[17] Cao W, Li Y, Duan LR, Liu YY, Wang K and Wang SW. Research of toad skin medicine quality standard. Shaanxi J Trad Chin Med 2012; 33: 1539-1541.

[18] Yan ZP, Peng GY, Zhang L, Zhang L and Zhang L. Multi-index Comprehensive Evaluation of the Extraction Technology of Liposoluble Constituents from the Skin of Bufo bufo gargarizans Cantor. Pharm J Chin PLA 2012; 28: 400-403.

[19] Cao XT, Wang D, Wang N, Dai YH and Cui Z. Isolation and identification of bufogenin constituents from the skin of Bufo bufo gargarizans Cantor. J Shenyang Pharm Univ 2009; 26: 778-781.

[20] Zhao DZ, Chen JY, Qin Y and Jiang YT. A comparison study on the chemical constituents of Venenum Bufonis and the skin of toad. Tianjin Pharm 2006; 18: 21-24.

[21] Wang YQ, Yan JY and Yu LH. Chemical composition and clinical application research progress of toad skin. Lishizhen Med and Mate Med Res 2009; 20: 1213-1214.

[22] Gao HM, Wu XY, Li ZY, You Y, Zhang Y and Wang ZM. Chemical constituents from Bufonis periostracum and their antitumor activity in vitro. China J Chin Mater Med 2011; 36: 2207-2210.

[23] Miao ZL, Zhang K, Yang MZ and Zhou XJ. Toad skin and fat toad exuviate toad venom ligands content determination and comparison. Lishizhen Med and Mate Med Res 2010; 21: 879-880.

[24] Xu LC. Toad clothing content of medicinal herbs in the fat toad venom ligands. Shandong J Tradit Chin Med 2008; 27: 624-625.

[25] Cerri A, Serra F, Ferrari P, Folpini E, Padoani G and Melloni P. Synthesis, cardiotonic activity, and structure-activity relationships of 17 beta-guanylhydrazone derivatives of 5 beta-androstane-3 beta, 14 beta-diol acting on the Na+,K(+)-ATPase receptor. J Med Chem 1997; 40: 3484-3488.

[26] Bick RJ, Poindexter BJ, Sweney RR and Dasgupta A. Effects of Chan Su, a traditional Chinese medicine, on the calcium transients of isolated cardiomyocytes: cardiotoxicity due to more than Na, K-ATPase blocking. Life Sci 2002; 72: 699-709.

[27] Xie JT, Dey L, Wu JA, Lowell TK and Yuan CS. Cardiac toxicity of resibufogenin: electrophysiological evidence. Acta Pharmacol Sin 2001; 22: 289-297.

[28] Xin XL, Zhang BJ, Su DH, Deng S, Huang SS and Ma XC. Pharmacology Advances in Studies on Bufo Bufo Gargarizans. Prog in Mod Biomed 2012; 12: 588-590, 600.

[29] Kamano Y, Yamashita A, Nogawa T, Morita H, Takeya K, Itokawa H, Segawa T, Yukita A, Saito K, Katsuyama M and Pettit GR. QSAR evaluation of the Ch'an Su and related bufadienolides against the colchicine-resistant primary liver carcinoma cell line PLC/PRF/5(1). J Med Chem 2002; 45: 5440-5447.

[30] Kamano Y, Kotake A, Hashima H, Inoue M, Morita H, Takeya K, Itokawa H, Nandachi N, Segawa T, Yukita A, Saitou K, Katsuyama M and Pettit GR. Structure-cytotoxic activity relationship for the toad poison bufadienolides. Bioorg Med Chem 1998; 6: 1103-1115.

[31] Chen XY, Xu RC, Chen L and Qian J. Studies on the Cytotoxicity of Bufalin on Human Gastric Cancer Cell M Gc-803 in vitr. Chin Tradit and Herb Drug 2000; 31: 920-922.

[32] Yin PH, Li Q, Qin AJ, Wang Y, Liu NN, Sui H, Li XQ and Wu Q. Study on Bufalin's Inducing Human Pancreatic Cancer Apoptosis and Its Mechanisms. Shanghai J Tradit Chin Med 2008; 42: 78-81.

[33] Su YH, Yin XC, Xie JM, Gao B and Ling CQ. Inhibition effects of three kinds of bufotoxins on human SMMC-7721 and BEL-7402 hepatoma cells lines. Aca J Sec Mili Med Univ 2003; 24: 393-395.

[34] Qu JL, Pan WL, Zhao LL, Sun L, Wang XP and Chen TS. Analyzing caspase-3 activation induced by bufalin using fluorescence techniques in single living cell. J Shenzhen Univ 2008; 25: 244-247.

[35] Jiang Y, Zhang Y, Luan J, Duan H, Zhang F, Yagasaki K and Zhang G. Effects of bufalin on the proliferation of human lung cancer cells and its molecular mechanisms of action. Cytotechnology 2010; 62: 573-583.

[36] Qiao L, Huang YF, Cao JQ, Zhou YZ, Qi XL and Pei YH. One new bufadienolide from Chinese drug "Chan'Su". J Asian Nat Prod Res 2008; 10: 233-237.

[37] Nogawa T, Kamano Y, Yamashita A and Pettit GR. Isolation and structure of five new cancer cell growth inhibitory bufadienolides from the Chinese traditional drug Ch'an Su. J Nat Prod 2001; 64: 1148-1152.

[38] Su CL, Lin TY, Lin CN and Won SJ. Involvement of caspases and apoptosis-inducing factor in bufotalin-induced apoptosis of Hep 3B cells. J Agric Food Chem 2009; 57: 55-61.

[39] Tian HY, Luo SL, Liu JS, Wang L, Wang Y, Zhang DM, Zhang XQ, Jiang RW and Ye WC. C23 steroids from the venom of Bufo bufo gargarizans. J Nat Prod 2013; 76: 1842-1847.

[40] Moreno YBL, Urban E, Gelbcke M, Dufrasne F, Kopp B, Kiss R and Zehl M. Structure-activity relationship analysis of bufadienolide-induced in vitro growth inhibitory effects on mouse and human cancer cells. J Nat Prod 2013; 76: 1078-1084.

[41] Liu J, Zhang D, Li Y, Chen W, Ruan Z, Deng L, Wang L, Tian H, Yiu A, Fan C, Luo H, Liu S, Wang Y, Xiao G, Chen L and Ye W. Discovery of bufadienolides as a novel class of ClC-3 chloride channel activators with antitumor activities. J Med Chem 2013; 56: 5734-5743.

[42] Watabe M, Masuda Y, Nakajo S, Yoshida T, Kuroiwa Y and Nakaya K. The cooperative interaction of two different signaling pathways in response to bufalin induces apoptosis in human leukemia U937 cells. J Biol Chem 1996; 271: 14067-14072.

[43] Liu Y, Qu X, Wang P, Tian X, Luo Y, Liu S and Lu X. WT1 downregulation during K562 cell differentiation and apoptosis induced by bufalin. Zhonghua Xue Ye Xue Za Zhi 2002; 23: 356-359.

[44] Takai N, Ueda T, Ishii T, Kira N, Nishida M, Nishida Y, Nasu K and Narahara H. Effects of bufalin on the proliferation of human choriocarcinoma cells. Int J Gynecol Cancer 2011; 21: 1105-1109.

[45] Takai N, Ueda T, Nishida M, Nasu K and Narahara H. Bufalin induces growth inhibition, cell cycle arrest and apoptosis in human endometrial and ovarian cancer cells. Int J Mol Med 2008; 21: 637-643.

[46] Huang C, Chen A, Guo M and Yu J. Membrane dielectric responses of bufalin-induced apoptosis in HL-60 cells detected by an electrorotation chip. Biotechnol Lett 2007; 29: 1307-1313.

[47] Wang J, Yin JQ, Jia Q, Shen JN, Huang G, Xie XB and Zou CY. [Bufalin induces apoptosis in osteosarcoma U-2OS and U-2OS methotrexate 300-resistant cell lines in vitro]. Zhonghua Zhong Liu Za Zhi 2010; 32: 734-738.

[48] Qi F, Inagaki Y, Gao B, Cui X, Xu H, Kokudo N, Li A and Tang W. Bufalin and cinobufagin induce apoptosis of human hepatocellular carcinoma cells via Fas- and mitochondria-mediated pathways. Cancer Sci 2011; 102: 951-958.

[49] Sun L, Chen T, Wang X, Chen Y and Wei X. Bufalin Induces Reactive Oxygen Species Dependent Bax Translocation and Apoptosis in ASTC-a-1 Cells. Evid Based Complement Alternat Med 2011; 2011: 249090.

[50] Kurosawa M, Tani Y, Nishimura S, Numazawa S and Yoshida T. Distinct PKC isozymes regulate bufalin-induced differentiation and apoptosis in human monocytic cells. Am J Physiol Cell Physiol 2001; 280: C459-464.

[51] Li H, Wang P, Gao Y, Zhu X, Liu L, Cohen L, Meng Z and Yang P. Na+/K+-ATPase alpha3 mediates sensitivity of hepatocellular carcinoma cells to bufalin. Oncol Rep 2011; 25: 825-830.

[52] Qi F, Li A, Inagaki Y, Kokudo N, Tamura S, Nakata M and Tang W. Antitumor activity of extracts and compounds from the skin of the toad Bufo bufo gargarizans Cantor. Int Immunopharmacol 2011; 11: 342-349.

[53] Miao Q, Bi LL, Li X, Miao S, Zhang J, Zhang S, Yang Q, Xie YH, Zhang J and Wang SW. Anticancer Effects of Bufalin on Human Hepatocellular Carcinoma HepG2 Cells: Roles of Apoptosis and Autophagy. Int J Mol Sci 2013; 14: 1370-1382.

[54] Xie CM, Chan WY, Yu S, Zhao J and Cheng CH. Bufalin induces autophagy-mediated cell death in human colon cancer cells through reactive oxygen species generation and JNK activation. Free Radic Biol Med 2011; 51: 1365-1375.

[55] Lee DY, Yasuda M, Yamamoto T, Yoshida T and Kuroiwa Y. Bufalin inhibits endothelial cell proliferation and angiogenesis in vitro. Life Sci 1997; 60: 127-134.

[56] Wang NY, Li SY, Zhao W, Qin SK, Liu L and Chen HY. Study the effect of antiangiogenesis of arsenic trioxide in combination with cinobufacin on chick embryo chorioallantoic membrane (CAM). Chin Clin Onco 2006; 11: 494-496.

[57] Zhang W, Liu YL, Xu CY, Gao E and Bao WF. Research on the Active Analgesic Component of Venenum Bufonis. J Shenyang Pharm Univ 1998; 15: 268-271.

[58] Watabe M, Ito K, Masuda Y, Nakajo S and Nakaya K. Activation of AP-1 is required for bufalin-induced apoptosis in human leukemia U937 cells. Oncogene 1998; 16: 779-787.

[59] Wei XL, Wang XY and Tang W. Effect of cinobufotalin on the dendritic cells derived from peripheral blood of health adults. J Nantong Univ (Med Sci) 2007; 27: 98-100.

[60] Chen CF, Miu J, Li JH, Zheng WF and Zou SC. Effect of Extraction from Toad Cake, Toad-cortex and Bufonic on ATP Enzyme of Myocardium Membrane. Sichuan J Zoo 2008; 27: 393-395.

[61] Cao L, Mao CP and Gu ZL. Experimental Study on the Sugar reducing Function of Cicada coat Powder. Chin J Hemorheo 2002; 12: 290-292.

[62] Chen JW, Li X, Xu JG and Zhou ZD. Experimental study on anti HIV-1 activity of Periostracum Bufonis of new resource of Chinese material medica. J US-China Med Sci 2007; 4: 20-22.

[63] Liang Y, Li P, Yu BY and Guo DA. Gastrointestinal Absorption and Biotransformation of Bufadienolides in Rats by HPLC/MS. Chin J Nat Med 2007; 5: 285-288.

[64] Arena JM and Drew RH. Poisoning: Toxicology, Symptoms Treatments. In: Tomas CC, editors. 5th. IL: Spring field; 1985. pp. 562-564.

[65] He SL, Ji AG and Peng ZP. Experimental study of anisodamine against toad venom toxicity. Pharm and Clin Chin Mater Med 1985; 203-205.

[66] Li XP, Lei L, Hu JY, Liu JY and Deng WL. Toxicity studies of toad venom and Danlingxinshu capsule. Pharm and Clin Chin Mater Med 2012; 28: 127-129.

[67] Jiangsu new medical college. Traditional Chinese medicine dictionary. Shanghai: Shanghai science and technology press; 1979.

[68] JIn QQ, Gu LL and Xie QX. The toxicity of toad skin preparations, safety test and the study of the central nervous system function. Bengbu Med J 1982; 7: 167-171.

[69] Yu CL and Hou HM. Bioassay-guided separation and acute toxicity evaluation of antitumor ingredient in Bufonis Venenum. Chin Tradit and Herb Drug 2011; 42: 307-311.

[70] Yoshida S, Kamano Y and Sakai T. Studies on the surface anesthetic activity of bufadienolides isolated from ch' an su. Chem Pharm Bull (Tokyo) 1976; 24: 1714-1717.

[71] Zhong SH, Hu TJ, Hao LH, Zhao H and He JK. Acute and chronic toxicity studies of toad venom injection. Chin J Vet Med 2013; 49: 68-71.

[72] Zhang ZF. Care of toad venom injection intravenous drip. J Mod Med Health 2013; 29: 1565-1566.

[73] Qian F. 272 cases of hospitalized patients with tumor cinobufacini injection usage analysis. Strait Pharm J 2013; 25: 158-159.

[74] Jiang JJ, Zhou Q, Ma HY, You FQ, Duan JA and Ding AW. Study on Cardiotoxicity and Toxic Substance of Toad Venom in Isolated Guinea Pig Heart. Chin J Exp Tradit Med Form 2011; 17: 233-237.

[75] Gao RL and Shao YX. Clinical application of venenum bufonis. Chin J Clin Rehabil 2006; 10: 142-144.

[76] Tang J, Jin R and Ye Y. The application of toad venom preparations in terms of anti-tumor. Pharmaceuti monogra 2008; 17: 15-16.

[77] Zhang HQ, Yan ZF and Cheng JY. Clinical application and research status quo of toad venom. Clin J Med Offi 2012; 40: 478-479.

[78] Zhou Y, Ji AG and Song SL. Study on application of Venenum Bufonis. Chin J Biochem Pharm 2009; 30: 203-206.

[79] Yang LX, Zhao HY, Yuan SF, Li YJ, Bian BL and Wang HJ. Determination of Total Bufadienolides in Cinobufotalin Injection Using Ultraviolet Spectrophotometry. Chin J Exp Tradit Med Form 2013; 19: 87-89.

[80] Han HB and Chen JY. Recent Reaserch Progress of Antitumor Advances in the Studies on the Antitumor Effect and Mechanism of Cinobufacini. Chin J Can Biothera 2005; 12: 160-162.

[81] Wang SS, Zhai XF and Li B. Effect of Cinobufacini Injection on the Tumor Growth of Tumor Bearing Rats of Different Constitutions. Chin J Inte Tradit and West Med 2011; 31: 1101-1103.

[82] Zhang TS and Shan GZ. Continuous Transarterial Infusion with Cinobufotain Combined with Transhepatic Arterial Embolism in the Treatment of 32 Cases with Primary Hepatocellular Carcinoma. J Onco 2011; 17: 557-558.

[83] Chen N and Chen XP. Inhibition of Cinobufacini on the Production of IL-2 from Mouse Spleen Lymphocyte. Hera Med 2007; 26: 14-16.

[84] Enomoto A, Rho MC, Komiyama K and Hayashi M. Inhibitory effects of bufadienolides on interleukin-6 in MH-60 cells. J Nat Prod 2004; 67: 2070-2072.

[85] Li D, Qu X, Hou K, Zhang Y, Dong Q, Teng Y, Zhang J and Liu Y. PI3K/Akt is involved in bufalin-induced apoptosis in gastric cancer cells. Anticancer Drugs 2009; 20: 59-64.

[86] Zhu Z, Yu Y, Wang K, Li E, Liu Y and Liu Y. [Effect of Bufalin on proliferation and apoptosis of human non-small cell lung cancer A549 cell]. Zhongguo Fei Ai Za Zhi 2010; 13: 841-845.

[87] Zhu ZT, Jin B, Liu YP, Li YC, Lu XL, Tian X and Hou KZ. [Enhancement of all-trans retinoic acid-induced differentiation by bufalin in primary culture of acute promyelocytic leukemia cells]. Zhonghua Nei Ke Za Zhi 2006; 45: 314-317.

[88] Zhai XF, Lv X, Gu W, Song CC and Li B. A Preliminary Study on Anti-angiogenesis Effect of

Bufalin. Chin J Inform on Tradit Chin Med 2010; 17: 29-31.

[89] Qin ZH. Clinical observation of cinobufacini capsule treatment of advanced gastric cancer 48 cases. Inte Med China 2013; 8: 38-39.

[90] Cao J, Wang Y, Ge XG and Zhou J. Clinical observation on toad venom injection in combination with chemotherapy treatment of advanced malignant tumor. Liaoning J Tradit Chin Med 2005; 32: 36-37.

[91] Zhao JQ, Xing LQ, Li W, Zhang SP, Li GL and Qian HH. Clinical Study on Efficacy of Bufalin Injection Combined with Chemotherapy in the Treatment of Advanced Non Small Cell Lung Cancer. Lishizhen Med and Mate Med Res 2006; 17: 1604-1605.

[92] Shang AL, Deng B and Li CY. Toad venom injection treatment of advanced esophageal cancer with radiotherapy 31 cases. China Naturopa 2005; 13: 51-52.

[93] Wang XM. Toad venom injection chemotherapy treatment of advanced non-small cell lung cancer with 34 cases. China Forei Med Treat 2010; 29: 39, 41.

[94] Huang ZF, Shi ZY, Luo Y and Huang N. Clinical efficacy of Toad Venom injection combined with chemotherapy in treating 31 patients with medium and advanced gastric cancer. Hebei J Tradit Chin Med 2002; 24: 163-165.

[95] Qiu BL and Dong GL. Clinical analysis of cinobufacini injection treatment of advanced malignant tumor of 60 cases. China Mod Med 2010; 17: 85-85.

[96] Wang CJ, Chen QQ, Deng L, Lu QH and Liang J. Hepatic Artery Infusion of Cinobufotain for Treating Advance Hepatocellular Carcinoma. Chin J INte Tradit and West Med on Liv Dis 2001; 11: 5-7.

[97] Ma JL and Lu M. Clinical research on 109 cases of non-small cell Lung cancer treated by cinobutacini injection plus gemcitabine and cisplatin. J Tradit Chin Med 2011; 52: 2115-2118.

[98] Liu XH, Fu H, Zhu QH, Pan PS and Yang L. Cinobufacini injection combined with conventional therapy of liver cancer clinical research. Chin J Mod Drug Appl 2009; 3: 134-135.

[99] Zhang XY, Hu YH, Tang CG, Li YC, Sun Y, Yuan ZZ, Chen J and Zhong GC. Clinical research of cinobufacini auxiliary body gamma knife stereotactic treatment of primary liver cancer. The Chin J Clin Phy (Electri) 2011; 05: 1901-1905.

[100] Yang YY and Xiong YP. Clinical observation of toad venom Jieninggao treatment of 120 advanced cancer cases of severe pain. Chin West Med J 2006; 4: 58-60.

[101] Chen MX, Huang LZ, He YH, Zhang H and Cao JX. Clinical observation of complex toad venom powder topical treatment of 60 cases of pain. J Hunan Coll Tradit Chin Med 2004; 24: 37-39.

[102] Liu JX. Clinical observation for the toad venom paste treatment of 332 cases of malignant tumor pain. J Tradit Chin Med 1988; 29: 30-31.

[103] Li J. Application of the Chanwu cataplasm treatment for patients with advanced lung cancer pain. Shanghai Nur 2007; 7: 24-25.

[104] Deng LR, Chen T, Gong ZP, Hu YQ and Yu XQ. Analgesic effect of the traditional Chinese medicine cinobufagin injection. Anhui Med and Pharm J 2010; 14: 397-399.

[105] Gong ZP and Chen T. Studies on analgesic mechanism based on opioid receptor for cinobufagin injection. Chin J Exp Tradit Med Form 2010; 16: 120-122.

[106] Huang M and Jiang SY. Clinical analysis of toad venom injection treatment of high fever suspected influenza A (H1N1). Chin J Misdiagn 2010; 10: 2079-2080.

[107] Xu XT and Jing PT. Clinical observation on cinobufacini treatment of chronic hepatitis B carriers. Chin J Inte Tradit and West Med 1993; 13: 473-475.

[108] Pan YR, Wu NP. Cinobufacini therapy for AFP continued anomaly of 28 cases of chronic hepatitis B. Zhejiang Clin Med J 2001; 3: 555-556.

[109] Liu H, Zhang PF, L DX, Ma Y and Tang GM. Clinical observation of monosodium phosphate vidarabine joint cinobufacini treatment of chronic hepatitis B. Chinese J Exp Clin Virol 2006; 20: 29.