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

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
Toad medicine

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


Figure 2. Chemical construction of Bufotaline.
Toad medicine

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 cino-bufagin (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_{6}, 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 garinizans of toad venom, and the concentration of of RBG, CBG, BL, and 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]. Watersoluble 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_{34}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].
Toad medicine

![Figure 5. Toad ring amide compounds structures. A. Bufogargarizaine B. Bufogargarizaine D. C. Bufogargarizaine C. D. Telocinobufagin. E. Desacetylcinobufotalin. F. Hellebrigenin.](image)

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$_{10}$H$_{12}$N$_{2}$O$_{4}$, *bufo gargarizans*-B, BGZ-B, Figure 5A) [13], the toad ring amide D (C$_{16}$H$_{26}$N$_{4}$O$_{9}$, *bufo gargarizaine*-D, BGZ-D, Figure 5B) [14], and exclude lactone structure compounds, such as toad ring amide C (C$_{24}$H$_{34}$O$_{5}$, *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 Borbou) from Taiwan and Bufo Bufo gargarizans (B. Bufo gargarizans 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 L-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) cholesteryl, 3) 5-alpha, 8-alpha-epidioxychol-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) delta3,5(E), delta3,15(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 Ca2+ 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 IC50 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 (IC50 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 (IC50 = 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 Xiaooy 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 μM. 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 [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 argenteogenin 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 CIC-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 WT1 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 GO/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 kinases 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 mem-
brane 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 relat-
ed to apoptosis signaling pathways, thus, event-
ually 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 hepatocel-
lar 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 invol-
ves 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
mmol/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 con-
tained the BL component in the chicken embryo allantois membrane and had an inhibi-
tory 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 analge-
sic 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 cen-
tral 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 anti-
bacterial 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 cap-
illary permeability during inhibition, reduces
medicinal overflow, and is beneficial in eliminat-
ing 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 S180 sarco-
ma, H22 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 pro-
duced 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 mix-
ture 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
Toad medicine

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 aglucce by rapid intravenous injection was 4.25 mg/kg, by slow intravenous injection was 15 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 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, tuberculosis 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, aero-sols, 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 masti (approval number: Z20063321), Tianchan capsule (approval number: Z20020056), the toad venom Juxin 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, respiratory, 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), Cinobufacin 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 1 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
Toad medicine

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 (H1N1). 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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Toad medicine


Toad medicine


Toad medicine


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