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Analgesic and toxic effects of *venenum bufonis* and its constituent compound cinobufagin: A comparative study

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ABSTRACT

Objective: In this study, we aimed to compare the analgesic and toxic effects of *Venenum Bufonis* (VB) with those of cinobufagin (CBG), a monomer isolated from VB, to provide the experimental basis for further research and development of VB.

Methods: After intragastric administration, the analgesic activities of VB and CBG were compared using the hot plate test and acetic acid-induced writhing test. Their side effects were also compared using hepatotoxicity, acute toxicity, cytotoxicity, and hemolytic toxicity tests, as well as by evaluating their effects on rat heart rate.

Results: In the hot plate test, both drugs prolonged paw withdrawal latency; however, CBG-treated mice exhibited significantly longer latency than VB-treated mice. In the acetic acid-induced writhing test, both drugs inhibited mouse writhing; however, the inhibitory effects of CBG were stronger. In addition, VB significantly increased serum aspartate aminotransferase (AST) levels, whereas CBG did not these levels. The LD_{50} of VB and CBG was 36.25 and 4.78 mg/kg, respectively. Both drugs increased the heart rate with CBG exhibiting stronger effects. Moreover, results showed that the cytotoxicity of CBG was more dose-dependent than that of VB. Both VB and CBG showed low hemolytic toxicity.

Conclusions: Both VB and CBG exhibited analgesic effects and low hemolytic toxicity; however, the latter showed stronger analgesic activity and less hepatotoxicity. Additionally, both VB and CBG increased the heart rate; however, CBG had stronger effects and higher acute toxicity.

1. Introduction

Venenum bufonis (VB), the white mucous substance secreted from the parotid and cutaneous glands of *Bufo bufo gargarizans* Cantor or *Bufo melanostictus* Schneider, is a precious traditional Chinese medicine produced mainly in the eastern and southern provinces of China. In traditional Chinese medicine, VB has been used for detoxification, detumescence, refreshment, and pain relief (Cao and Shibayama-Imazu, 2007). The Chinese medicinal monographs "Ben Cao Hui Yan" report that VB can cross the 12 meridians, viscera, interpleuro-diaphragmatic space, muscular interstice, and the joints. It is used to treat malnutritional stagnation, tympanites, and furunculosis; additionally, it can reduce congestion and stagnation, including poison accumulation, mass accumulation, distention, and internal carbuncle (Xiao and Xue, 2014). However, VB has a complex chemical composition, making it difficult to carry out in-depth research and development. Cinobufagin (CBG) is the main active component in VB, and as a monomer, it has clear molecular and structural formulas. Basic and clinical studies have shown that the pharmacological activity of CBG is similar to that of VB (Wang et al., 1998; Zhang and Liu, 2012; Bi and Hou, 2016).

VB contains various compounds, including polypeptides, steroids, indole alkaloids, and organic acids. Steroids isolated from VB include bufadienolides and cholesterol (Chang and Li, 2018). Most VB-derived chemicals with a defined chemical composition are bufadienolide complexes, such as CBG. Bufadienolides are widely recognized as the main active components of VB; however, VB also shows varying degrees of toxicity (Wang et al., 2014; Dai and Zheng, 2018). In this study, we aimed to compare the analgesic and toxic effects of VB and CBG and to evaluate the efficacy and safety of VB, thus providing the experimental basis for future research and use of VB.

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2. Materials and methods

2.1. Experimental animals

Kunming mice (20 ± 2 g) and Sprague-Dawley rats (200 ± 20 g) were purchased from Qinglongshan Animal Breeding Center (Nanjing, China). They were housed at 22 ± 1 °C and 60 ± 5% humidity with a 12-h light-dark cycle. They were fed *ad libitum* and acclimatized in a non-stressful environment for at least 1 week prior to the experiments. Animal experiments were performed in accordance with the Guide for the Care and Use of Laboratory Animals of China Jiaxing University [Animal use protocol no: SCXK (HU) 2017–0001] and approved by the Ethics Committee of Animal Experimentation of Jiaxing University. All laboratory procedures were carried out with the permission and under the surveillance of the ethics committee.

2.2. Reagents and instruments

VB was kindly provided by Dong-fang Guo-yao Co., Ltd. (Jiaxing, China). The content of the CBG in VB was 4.67%. CBG (batch number: BCTG-0290) was provided by the National Pharmaceutical Engineering Center (Nanchang, China). Morphine hydrochloride (batch number: 1448016) and aspirin (batch number: A2093) were purchased from Sigma (MO, USA). Other reagents and instruments included 0.6% glacial acetic acid solution, an automatic biochemical analyzer (COBAS Integra 800, Roche, Switzerland), a hot plate pain threshold detector (Shanghai Yuyan Scientific Instrument Co., Ltd., China), and a multifunction animal monitor (Shanghai Yuyan Scientific Instrument Co., Ltd.).

2.3. Preparation of VB and CBG

VB and CBG (2 g each) were soaked in 10× volume of water for injection, ground, mixed with ethanol until alcohol concentration reached 75%, stirred, refrigerated, and filtered. The filtrate was concentrated until ethanol was completely removed, mixed with a certain volume of water for injection, stirred, refrigerated, and filtered. Then, anhydrous sodium sulfite (2 g), sodium chloride (8 g), and benzyl alcohol (10 mL) were added, and the mixture was stirred until dissolution. The pH was adjusted using 40% sodium hydroxide, the volume was completed to 1000 mL with water for injection, and the solution was stirred, filtered, sealed, and sterilized.

2.4. Evaluation of analgesic effects

Animals were divided into 4 groups: CBG (2 mg/kg), VB (2 mg/kg), vehicle (CBG and VB vehicles), and morphine (3 mg/kg, subcutaneous, as a positive control) in the hot plate test or aspirin (300 mg/kg, as a positive control) in the writhing antinociceptive and formalin tests. CBG, VB, vehicle, and aspirin were administered *via* oral gavage. The dose was selected based on our preliminary screening.

2.4.1. Hot plate test

The hot plate temperature was adjusted to 55 ± 0.5 °C, and the mice were placed on it. The response of licking the hind paw was closely observed as a pain sensitivity index. The reaction time from placement to licking or biting the hind paws or jumping was recorded as an index of pain threshold (Zhang et al., 2006a, 2006b). Mice with mild hyperalgesia (jumping, escaping, or pain threshold < 10 s) and unresponsive mice (pain threshold > 30 s) were excluded. Qualified experimental mice were screened. The latency was recorded 30 min before administration (baseline latency) and 15, 30, 60, and 90 min after administration. If mice still had no pain reaction within 60 s, the pain threshold was considered 60 s. The percentage of pain threshold elevated rate (PTE%) was calculated using the following formula: PTE (%) = 100 × (latency in experimental group – latency in vehicle control)/latency in vehicle control.

2.4.2. Acetic acid writhing test

Mice in different groups were pretreated with the drug for 0.5 h before injection of 0.6% acetic acid solution (10 mL/kg, intraperitoneal [i.p.]). The total number of abdominal constrictions was recorded from 5 to 20 min after acetic acid injection (Zhang et al., 2006a, 2006b). The percentage of inhibition was calculated according to the following formula: Inhibition (%) = 100 × (number in vehicle control − number in experimental group)/number in vehicle control.

2.5. Serum hepatic enzyme levels

The levels of serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were measured in mice treated with the vehicle [intragastric (i.g.)], VB (2 mg/kg, i.g.), CBG (2 mg/kg, i.g.), and CBG (4 mg/kg, i.g.) after 24 h. Blood was collected and stored on ice before centrifugation at 1000 ×*g* at 4 °C for 10 min. Then, the serum was collected. The levels of ALT and AST were measured using assay kits (Nanjing Jiancheng Bioengineering Institute, China). The absorbance of the reaction mixture was measured using a spectrophotometer at 505 nm for both ALT and AST.

2.6. Heart rate measurement

Twenty-four male rats were randomly divided into three groups: CBG (2 mg/kg, i.g.), VB (2 mg/kg, i.g.), and vehicle (i.g.) groups. The rats were anesthetized with pentobarbital sodium, and the heart rate was recorded using a multifunctional animal monitor (Shanghai Yuyan Instruments, China) 30 min before administration and 15, 30, 60, and 90 min after i.p. drug administration.

2.7. Toxicity assays

The toxicity of VB and CBG was evaluated using the mouse i.p. median lethal dosage (LD_{50}) method (Ng'uni and Klaasen, 2018). A total of 130 mice (65 male and 65 female) were randomly assigned into 13 subgroups, with 7 subgroups in the VB group and 6 subgroups in the CBG group. Mice in the VB subgroups were treated with 50, 42, 39, 36, 33, 30, or 20 mg/kg VB, whereas those in the CBG subgroups were treated with 15, 10, 7.5, 5, 2.5, and 1.25 mg/kg CBG.

After i.p. administration, acute toxicity and death were observed in each subgroup, and all mice were euthanized after 14 days. LD_{50} was calculated using the Bliss method. Mice that died during the test or were euthanized were anatomized for observation.

2.8. Cytotoxicity assay

Cell viability was assessed using the MTT assay. BRL cells (1×10^4/mL) were added to a 96-well culture plate (100 µL in each well), treated with CBG or VB at 0, 50, 100, 200, 400, 800 µg/mL for 48 h. The MTT reagent (5 µg/mL) was added to the wells and incubated for 4 h. The crystals produced were dissolved with formazan, and the absorbance of each sample was measured at 570 nm. The inhibition rate was calculated according to the following formula: Rate of inhibition = (control group − treatment group) / control group.

2.9. Hemolytic toxicity assay

Heparin-stabilized human blood was freshly collected and used within 3 h. Blood was washed with phosphate-buffered saline (PBS), centrifuged, and the white blood cells were removed until the supernatant was not red. The supernatant was discarded, and 1 mL of red blood cells was diluted to 50 mL with PBS to obtain a 2% red blood cell suspension. The concentrations of VBG and VB were adjusted to 25, 50, 100, 150, and 200 µg/mL with PBS. CBG or VB (0.5 mL) at different

concentrations was mixed with 0.5 mL of 2% red blood cell suspension, allowed to stand at 25 °C for 3 h, and then centrifuged at 10,050 rpm for 3 min. Then, 100 μL of each mixture was transferred to a 96-well plate and detected at a wavelength of 570 nm. Pure water and PBS were used as positive and negative controls, respectively. The percent of hemolysis was calculated as follows: % Hemolysis = [(sample absorbance − negative control absorbance) / (positive control absorbance − negative control absorbance)] × 100.

2.10. Data analysis

SPSS 15.0 statistical software for Windows was used for statistical analyses. Data were presented as the means ± standard error of the mean. All data were analyzed using one-way analysis of variance (ANOVA) or repeated-measures ANOVA. A P value < 0.05 was considered statistically significant.

3. Results

3.1. Evaluation of analgesic effects

3.1.1. Hot plate test

Both CBG (2 mg/kg) and VB (2 mg/kg) considerably increased the latency time of licking or jumping, compared to that in the control group. The antinociceptive effects were maintained for 15–90 min and reached the maximum after 30 min. The peak PTE was 23.8% and 14.1% after 30 min for CBG and VB, respectively. The antinociceptive effects of morphine (3 mg/kg) peaked after 30 min, and PTE was 28.6%. The mice injected with CBG and morphine hydrochloride showed longer time of paw withdrawal than that in the VB group. Repeated-measures ANOVA showed significant differences among groups ($P < 0.05$, $n = 8$; Fig. 1A). Additionally, the inhibitory effects of both CBG and VB were dose- and time-dependent.

3.1.2. Acetic acid writhing test

Injection of 0.6% acetic acid (10 mL/kg) induced abdominal constrictions in the vehicle group. Administration of CBG and VB significantly reduced the number of abdominal constrictions by 66.3 and 41.7%, respectively, compared to that in the control group ($P < 0.05$, $n = 11$; Fig. 1B). Aspirin (300 mg/kg) reduced the number of abdominal constrictions by 73.5%. The inhibitory effects of both CBG and VB were also dose-dependent, and CBG exerted stronger inhibitory effects than those of VB.

3.2. Hepatotoxicity of VB and CBG in rats

Serum ALT and AST levels were measured to evaluate the toxic effects of VB and CBG on rat liver 12 h after i.g. administration. As shown in Fig. 2A and B, the levels of ALT and AST in rats treated with CBG at two doses were not significantly different from those in the control ($P > 0.05$, $n = 8$), whereas AST level in VB-treated rats was significantly higher than that in the control ($P < 0.05$, $n = 8$), indicating greater hepatotoxic effects of VB than that of CBG.

3.3. Effects of VB and CBG on rat heart rate

The heart rate of rats was measured at 15, 30, 60, and 90 min after i.g. administration of VB, CBG, or normal saline. As shown in Fig. 3, the heart rate of VB- and CBG-treated rats significantly increased, compared to that in vehicle-treated rats ($P < 0.05$, $n = 8$). CBG-treated rats exhibited a significantly higher heart rate at 30, 60, and 90 min after administration than that of VB-treated rats ($P < 0.05$, $n = 8$; Fig. 3). These results suggested that both VB and CBG increased the heart rate in rats; however, CBG exhibited stronger effects.

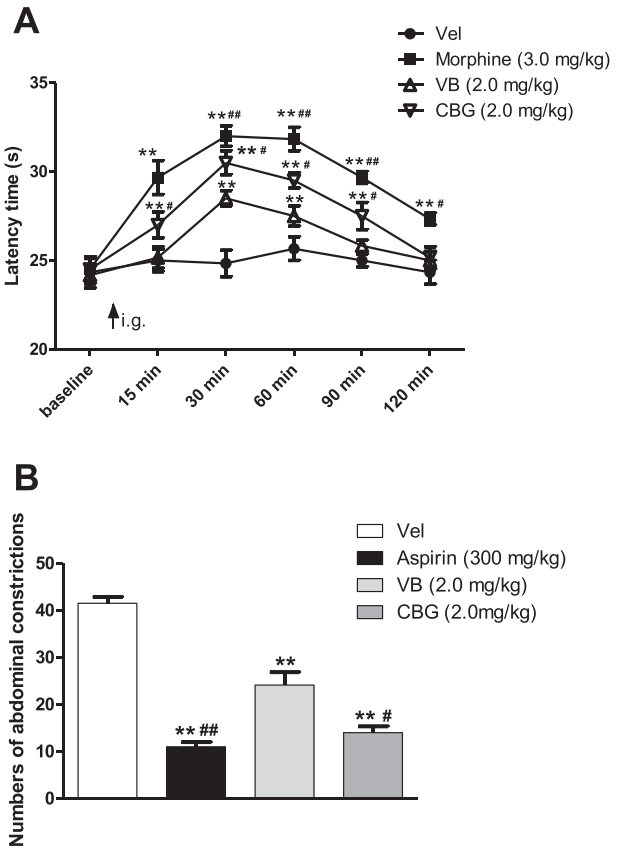

Fig. 1. Comparison between the analgesic effects of VB and CBG. Mice were treated with the vehicle, CBG (2 mg/kg), VB (2 mg/kg), morphine (3 mg/kg), or aspirin (300 mg/kg). (A) Analgesic effects of CBG and VB in the hot plate test. The latency time was measured 30 min before and 15, 30, 60, 90, and 120 min after administration. (B) Analgesic effects of CBG and VB in the acetic acid writhing test. The number of abdominal constrictions was recorded from 5 to 20 min after acetic acid injection. All data represent the means ± standard error of the mean ($n = 8$ mice/group). $^{*}P < 0.05$, $^{**}P < 0.01$ compared to the vehicle-treated group; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ compared to the VB-treated group.

3.4. Acute toxicity of VB and CBG in mice

After i.p. administration of drugs, mice showed eye closing, curling up, breath inhibition, and decreased activity. Death from VB intoxication occurred mainly 20–120 min after administration, whereas death from CBG intoxication occurred 10–60 min after administration. Convulsions and twitches occurred before death. The LD_{50} of VB and CBG was 36.25 and 4.78 mg/kg, respectively.

3.5. Acute cytotoxicity of VB and CBG in BRL cells

Cytotoxicity of VB and CBG at various concentrations (50, 100, 200, 400, and 800 μg/mL) was measured in BRL cells. As shown in Fig. 4, the cytotoxicity of CBG at 50 and 100 μg/mL was significantly higher than that of VB ($P < 0.01$, $n = 3$; Fig. 4). However, the cytotoxicity of VB at 200 μg/mL was significantly higher than that of CBG ($P < 0.01$, $n = 3$; Fig. 4) with no further increase in VB cytotoxicity at 400 and 800 μg/mL. However, the cytotoxicity of CBG increased in a concentration-dependent manner. These findings indicated that the cytotoxicity of CBG was more dose-dependent than that of VB.

3.6. Hemolytic toxicity of VB and CBG

The effects of VB and CBG on human red blood cells were evaluated

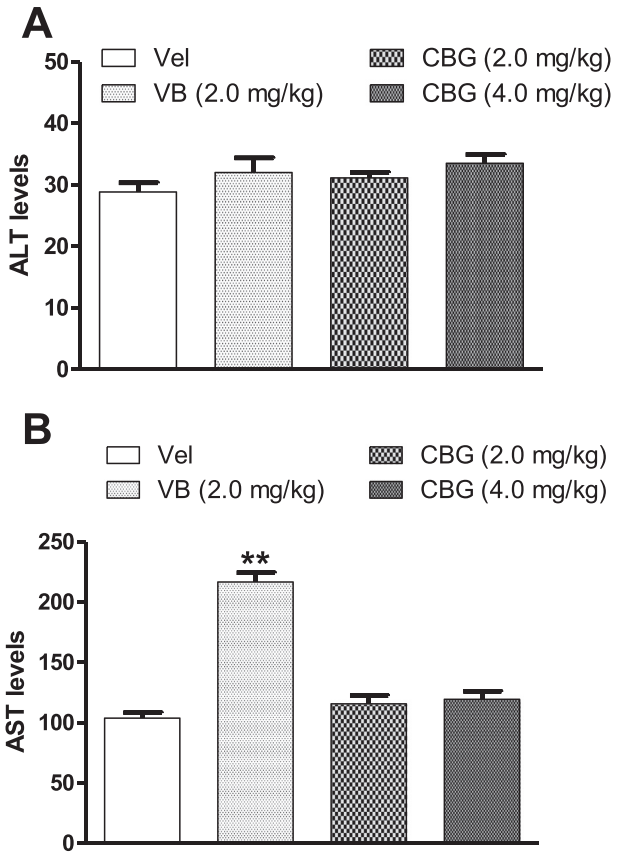

Fig. 2. Serum levels of ALT and AST at 24 h after administration of CBG (2 and 4 mg/kg, i.g.), VB (2 mg/kg, i.g.), or vehicle. (A) Serum levels of ALT were measured 24 h after administration of CBG (2 and 4 mg/kg, i.g.), VB (2 mg/kg, i.g.), or vehicle. (A) Serum levels of AST were measured 24 h after administration of CBG (2 and 4 mg/kg, i.g.), VB (2 mg/kg, i.g.), or vehicle. Data represent the means ± standard error of the mean ($n = 8$ mice/group). $^{**}P < 0.01$ compared to the vehicle-treated group.

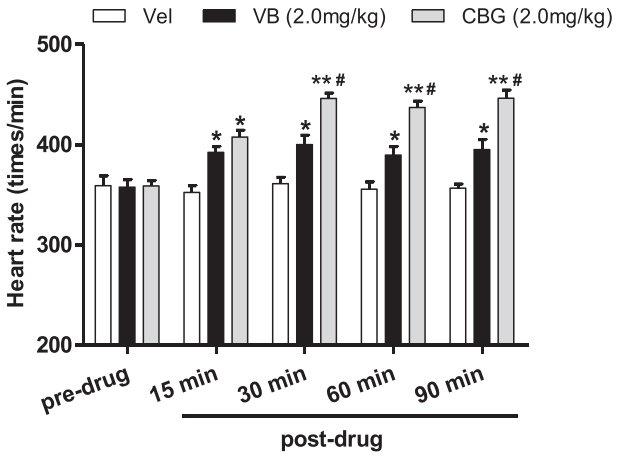

Fig. 3. Heart rate measurement before and 15, 30, 60, and 90 min after administration of CBG (2 mg/kg, i.g.), VB (2 mg/kg, i.g.), or vehicle. All data represent the means ± standard error of the mean (n = 8 mice/group). $^{*}P < 0.05$, $^{**}P < 0.01$ compared to the vehicle-treated group; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ compared to the VB-treated group.

using a hemolysis assay. As shown in Fig. 5, the hemolytic toxicity of VB and CBG increased in a dose-dependent manner, and the potency of both drugs was similar. Moreover, there was no significant difference in the hemolysis rate between VB and CBG ($P > 0.05$, $n = 3$; Fig. 5).

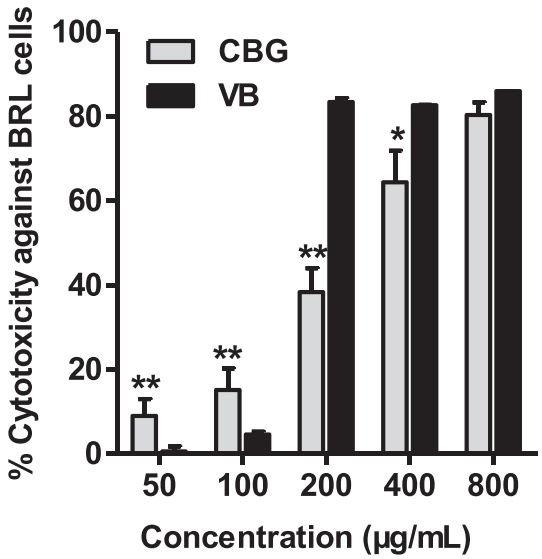

Fig. 4. Cytotoxicity to BRL cells at 48 h after treatment with CBG (50, 100, 200, 400, and 800 μg/mL) or VB (50, 100, 200, 400, and 800 μg/mL) using the MTT assay. All data represent the means ± standard error of the mean ($n = 3$). $^{*}P < 0.05$, $^{**}P < 0.01$ compared to the VB-treated group.

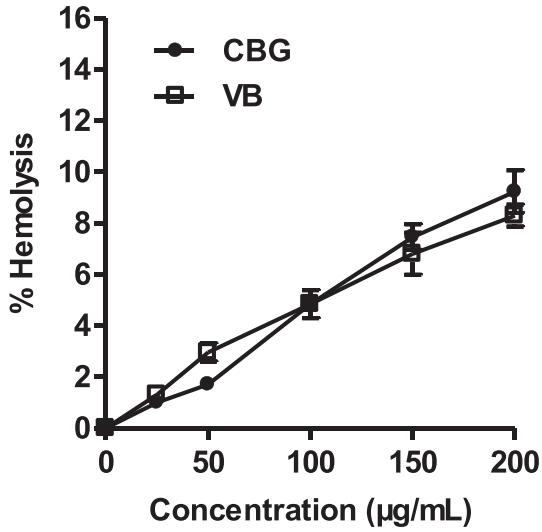

Fig. 5. Hemolysis after addition of CBG (25, 50, 100, 150, and 200 μg/mL) or VB (25, 50, 100, 150, and 200 μg/mL) on RBCs. Hemolysis was evaluated by measuring the absorbance of hemoglobin at 570 nm. All data represent the means ± standard error of the mean (n = 3).

4. Discussion

As a traditional Chinese medicine with complex chemical composition, VB has been used to decrease body temperature and for detoxification, refreshing the brain, and removing masses. VB has been shown to exhibit various pharmacological activities, where it can decrease blood pressure, enhance myocardial contractility, increase coronary blood flow, and protect against tumors. In clinical practice, VB is widely used for treating cardiovascular, respiratory, facial, surgical, and other diseases (Wang and Narui, 1991; Dong et al., 2015). Recently, VB has been found to relieve pain and detoxify the body; hence, it is used as an antitumor and analgesic agent. Since VB has strong local anesthetic effects, it is often used in clinics to treat different types of pain (Bi and Hou, 2016). Our study also showed that VB (2 mg/kg) significantly increased the latency time of licking or jumping in the hot plate test, compared to that in the control group. The antinociceptive effects were

maintained for 15–90 min and reached the maximum after 30 min. The peak PTE after 30 min was 14.1% at 2 mg/kg VB. Additionally, the inhibitory effects of VB were dose- and time-dependent. However, the analgesic effects of VB might be mediated by different active ingredients *via* action on multiple targets. Moreover, cardiac glycosides present in VB and its several side effects, such as arrhythmia and respiratory distress, limit the clinical use of VB despite its various beneficial pharmacological effects (Chen et al., 2014).

Owing to the complex chemical composition of VB, further studies on VB are particularly challenging. CBG, the main active component of VB, is a monomer with known molecular and structural formulae. Comparing the analgesic and toxic effects of CBG with those of VB is of great importance for in-depth exploitation and use of VB. Results of the current study showed that CBG (2 mg/kg) significantly increased the latency time of licking or jumping in the hot plate test, compared to that in the control group. The antinociceptive effects were maintained for 15–90 min and reached the maximum after 30 min. The peak PTE after 30 min was 23.8% at 2 mg/kg CBG. The antinociceptive action of morphine (3 mg/kg) peaked after 30 min, and the peak PTE was 28.6%. CBG- and morphine hydrochloride-treated mice showed longer paw withdrawal time than VB-treated mice. Zhang et al. showed that six fat-soluble active constituents in VB exerted analgesic effects in acetic acid-induced writhing and hot plate tests, among which CBG (1.3 mg/kg) showed the strongest effects (Wa et al., 1982). In addition, Chen et al. found that CBG significantly alleviated cancer pain in a lower limb cancer pain model (Chen and Hu, 2013).

VB has a narrow therapeutic index, and poisoning and allergy have been repeatedly reported in clinical practice. Systemic studies on the efficacy and side effects of VB could be performed with the improvement in modern molecular biology and experimental technology. In addition, optimized extraction techniques enabled the preparation of highly effective and less toxic drugs that could be safely applied in clinical practice. However, only few studies investigated the analgesic and toxic effects of VB. In the present study, we showed that the analgesic effects of CBG were more rapid than those of VB, where 15 min after treatment, VB had no analgesic effect, whereas CBG exerted significant analgesic effect comparable to that of morphine. The potency of CBG was also significantly higher than that of VB. However, VB resulted in hepatotoxicity as shown by the increased AST levels at 2 mg/kg, whereas CBG did not induce hepatotoxicity even at 4 mg/kg, indicating the therapeutic potential of CBG as an analgesic agent instead of VB. VB-induced hepatotoxicity might be attributed to the presence of CBG-unrelated impurities.

Our results indicated that both VB and CBG exhibited analgesic effects; however, the latter showed stronger effects and lower hepatotoxicity. Therefore, the use of CBG as an analgesic might be advantageous over VB as CBG had higher analgesic potency, faster onset time, and exhibited lower hepatotoxicity than VB.

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Author contributions

L.X. and M.Y. performed the experiments and analyzed the data; Y.Z., X.Z., H.S., Q.F., H.N., and H.S. interpreted the results; Q.F. and X.Z. prepared the figures; L.X. drafted the manuscript; L.X. and M.Y. edited and revised the manuscript; and M.Y. contributed to the study conception and design. All authors approved the final version of the manuscript.

Competing interests

There are no competing interests to declare.

References

Bi, Q. R., J. J. Hou, et al. (2016). "Venenum Bufonis induces rat neuroinflammation by activating NF-kappaB pathway and attenuation of BDNF." J. Ethnopharmacol. 186: 103–110.

Cao, H., T. Shibayama-Imazu, et al. (2007). "Involvement of Tiam1 in apoptosis induced by bufalin in HeLa cells." Anticancer Res. 27(1A): 245–249.

Chang, H., J. Li, et al. (2018). "Bufadienolides from Venenum Bufonis inhibit mTOR-mediated cyclin D1 and retinoblastoma protein leading to arrest of cell cycle in cancer cells." Evid. Based Complement. Alternat. Med. 2018: 3247402.

Chen, T., W. Hu, et al. (2013). "A study on the mechanism of cinobufagin in the treatment of paw cancer pain by modulating local beta -endorphin expression in vivo." Evid. Based Complement. Alternat. Med. 2013: 851256.

Chen, G., Jiang, X., et al., 2014. Study on anti-osteosarcoma activity of ethanol extract of Venenum Bufonis in vitro. Afr J Tradit Complement Altern Med 11 (1), 73–77.

Dai, G., D. Zheng, et al. (2018). "Cinobufagin induces apoptosis in osteosarcoma cells via the mitochondria-mediated apoptotic pathway." Cell. Physiol. Biochem. 46(3): 1134–1147.

Dong, G., Wei, D., et al., 2015. Study of the cardiotoxicity of Venenum Bufonis in rats using an 1H NMR-based metabolomics approach. PLoS One 10 (3), e0119515.

Ng'uni, T., J. A. Klaasen, et al. (2018). "Acute toxicity studies of the South African medicinal plant Galenia africana." Toxicol. Rep. 5: 813–818.

Wa, Z., Liu, Y., et al., 1982. Research on the active analgesic component of Venenum Bufonis. Journal of Shenyang Pharmaceutical University 15 (14), 268–271.

Wang, J. D., T. Narui, et al. (1991). "Hematological studies on naturally occurring substances. VI. Effects of an animal crude drug "chan su" (bufonis venenum) on blood coagulation, platelet aggregation, fibrinolysis system and cytotoxicity." Chem Pharm Bull (Tokyo) 39(8): 2135–2137.

Wang, J., Jin, W., et al., 1998. Determination of resibufogenin and cinobufagin in venenum Bufonis by HPLC. Zhongguo Zhong Yao Za Zhi 23 (11), 651–653 (702).

Wang, Z.J., Sun, L., et al., 2014. Resibufogenin and cinobufagin activate central neurons through an ouabain-like action. PLoS One 9 (11), e113272.

Xiao, Y., Xue, Q.F., 2014. Research on therapeutic effects of bufonis venenum on L7212 leukemia and its mechanism. Zhong Yao Cai 37 (2), 199–201.

Zhang, D.M., Liu, J.S., et al., 2012. Bufotalin from Venenum Bufonis inhibits growth of multidrug resistant HepG2 cells through G2/M cell cycle arrest and apoptosis. Eur. J. Pharmacol. 692 (1–3), 19–28.

Zhang, H.L., Han, R., et al., 2006a. Analgesic effects of receptin, a chemically modified cobratoxin from Thailand cobra venom. Neurosci. Bull. 22 (5), 267–273.

Zhang, H.L., Han, R., et al., 2006b. A short-chain alpha-neurotoxin from Naja naja atra produces potent cholinergic-dependent analgesia. Neurosci. Bull. 22 (2), 103–109.