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Original Research Article

Pharmacokinetic, acute toxicity, and pharmacodynamic studies of semen strychni total alkaloid microcapsules

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Abstract

Purpose: *To investigate the safety and effectiveness of semen strychni total alkaloid microcapsules (SSTAM), compared with semen strychni total alkaloids (SSTA).*
Methods: *Liquid chromatography-tandem mass spectrometry (LC-MS/MS) was employed to assess pharmacokinetics of brucine and strychnine in rats. Acute toxicity was investigated in pre-test and formal experiments in mice. The pharmacodynamics of SSTAM and SSTA were evaluated by their analgesic and anti-inflammatory activities.*
Results: *With respect to brucine, the half-life of SSTA group (1.6 mg/kg), low-dose SSTAM group (6 mg/kg) and high-dose SSTAM group (10 mg/kg) was 5.723, 9.321 and 9.025 h, respectively. With respect to strychnine, the half-life of SSTA group, low-dose SSTAM group and high-dose SSTAM group was 4.065, 8.819 and 8.654 h, respectively. The LD_{50} values of SSTAM group and SSTA group were 236.59 and 30.27 mg/kg, respectively. The pain inhibition rates of SSTAM groups (25 and 50 mg/kg) were higher than that of SSTA group ($p < 0.05$) while the pain threshold values of the SSTAM groups (25 and 50 mg/kg) were higher than that of blank control ($p < 0.01$) and SSTA groups ($p < 0.01$) at 60 min and 120 min. The inhibition rates of the SSTAM groups (25 and 50 mg/kg) were higher than that of SSTA group based on ear swelling and cotton ball granulation tests. Compared with blank control and SSTA groups, the absorbance values of SSTAM groups (25 and 50 mg/kg) were lower ($p < 0.01$).*
Conclusion: *SSTAM increases the dosage of administration but reducea the toxicity of the alkaloids in rats, and is thus a potentially safe and effective drug delivery system.*

Keywords: *Pharmacokinetics, Acute toxicity, Pharmacodynamic, Semen strychni, Total alkaloids, Microcapsules, Brucine, Strychnine*

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INTRODUCTION

Semen strychni is the dry ripe seed of *Strychnas nux-vomica L* [1]. Semen strychni is rich in alkaloids, including brucine, strychnine, brucine N-oxide, strychnine N-oxide, pseudo-brucine, pseudo-strychnine and icajine, which are active anti-tumor ingredients. In semen strychni total

alkaloids, brucine and strychnine are two major ingredients. Brucine has anti-tumor and anti-inflammatory effects, and is less toxic than strychnine. It has significant inhibitory and anti-angiogenic effects on liver cancer, lung cancer, colon cancer, breast cancer and multiple myeloma [2-6]. However, because the toxic dose is close to the effective dose, the therapeutic window is narrow, thus clinical application is limited.

Microencapsulation, a well-known technology for drug delivery, has drawn extensive research attention, recently [7-9]. Natural or synthetic macromolecule materials, which are employed as capsule wall materials, can also be used to prepare microcapsules. solid or liquid pharmaceutical preparations can both be packaged into microcapsules. Especially, the microencapsulation technology has been applied in the field of traditional Chinese medicine [10], though it is still at the laboratory research stage. In terms of slow drug release and low toxicity, the microencapsulation technology has promising potential in drug delivery.

Herein, we employed liquid chromatography-tandem mass spectrometry (LC-MS/MS) to assess the pharmacokinetics of brucine and strychnine in semen strychni total alkaloid microcapsules (SSTAM) and semen strychni total alkaloids (SSTA). Besides, based on an acute toxicity test, the dosage of SSTAM was confirmed. Furthermore, the pharmacodynamics of SSTAM was investigated, in comparison with SSTA.

EXPERIMENTAL

Reagents and animals

Semen strychni total alkaloid microcapsules (SSTAM) and semen strychni total alkaloids (SSTA) were prepared by the Pharmaceutical Preparation Laboratory of Guiyang College of Traditional Chinese Medicine. Brucine reference substance (lot no. 110706-201505, [purity ≥ 98 %]) and strychnine reference substance (lot no. 110706-201507, [purity ≥ 98 %]) were supplied by Beijing Shengshi Kangpu Chemical Technology Research Institute. Aspirin (lot no. 110606) was obtained from Shenyang Aohua Pharmaceutical Co. Ltd (Shenyang, China). Indomethacin reference substance (lot no. 100258-200904, 99.9 % purity) was provided by National Institute for Drug and Food Control. KunMing mice (KM mice, SPF grade, weight 20 ± 2 g, male-to-female ration = 1:1) and Sprague-Dawley rats (SD rats, SPF grade, weight 300 ± 50 g, male-to-female ratio, 1:1) were provided by the Institute of Experimental Animals (Guizhou Medical University). The quality certificate no. of the experimental animals was SCXK (Qian) 2012-0001. Prior to the study, approval was obtained from the Ethics Committee of Guiyang University of Chinese Medicine.

Drug administration and blood collection

Eighteen SD rats were used for pharmacokinetic study. They were divided into 3 groups randomly, based on intragastric administration: high-dose SSTAM group (10 mg/kg), low-dose SSTAM group (6 mg/kg) and SSTA group (1.6 mg/kg). The brucine dose (0.30 mg/kg) was same in SSTA group and low-dose SSTAM group and so was the strychnine dose (0.22 mg/kg). Blood was drawn from the orbital venous plexus of rats using glass capillaries at 15, 30, 60, 90, 120, 240, 360, 480, 600, 720 and 1440 min; 0.5 mL blood was drawn into 1.5 mL centrifuge tubes.

Plasma samples (200 µL) were obtained, contained in the tube with 10 µL of heparin sodium, which was as an anticoagulant. Afterwards, the samples mixed with 20 µL of methanol and 20 µL of internal standard solution (1080 ng/mL of indomethacin) and vortexed for 30 sec. Subsequently, 1 mL of chloroform was added and the samples were vortexed for 30 sec, subjected to ultrasound for 5 min, vortexed for another 30 sec and centrifuged at 5000 rpm for 5 min. The samples were dried by nitrogen at 37 °C, re-dissolved with 100 µL of methanol, vortexed for 1 min, subjected to ultrasound for 3 min and centrifuged at 13,000 rpm for 10 min. Eventually, the supernatant was taken out for injection analysis. The pharmacokinetic parameters were acquired by DAS software (V2.0).

LC-MS/MS analysis

The LC parameters were as follows: the chromatographic column was a Waters BEH C18 (2.1 mm × 50 mm, 1.7 µm); the mobile phase was 0.2 % formic acid/water-acetonitrile and the volume ratio of the mobile phase was in light of the following gradient elution: 0-0.4 min (60 % B→), 0.4- 0.6 min (60-78 % B→), 0.6-1.0 min (78 % B→), 1-1.5 min, (from 78-60 % B→) and 1.5-2 min (60% B); the flow rate was 0.35 mL/min; the column temperature was 40 °C. The MS parameters were set as follows: capillary ionization voltage, 3 KV; ion source temperature, of 120 °C; desolvation temperature, 550 °C; desolvation gas flow rate, 650 L/h; blowback gas flow rate, 50 L/h; cone voltage, 50 V, collision energy, 30 Ev; scanning mode, positive ion multi-reaction ion monitoring mode; quantitative ion of

brucine, m/z 395.27 → 244.11, 324.16; quantitative ion of strychnine, m/z 335.21 → 156.04,184.07; and quantitative ion of indomethacin, m/z 358.12 → 138.99, 110.96.

Acute toxicity studies

Forty healthy KM mice were raised for 1 week and divided into the following 10 groups (n = 4): SSTAM (50, 100, 200, 300, and 400 mg/kg); and SSTA (5, 15, 30, 40, 50 mg/kg). Mice received an intragastric administration of 0.2 mL/10 g, followed by continuous observation of mental status, appearance, activity, and other conditions for 2 weeks; deaths were recorded. Next, 130 KM mice were fasted for 12 h and randomly divided into 13 groups (n = 10), as follows: SSTA (6 dose groups); SSTAM (6 dose groups); and a saline control group.

The dose ratio of two adjacent groups was designed with a gradient of 1:0.8 and mice received an intragastric administration of 0.2 mL/10 g. The symptoms of poisoning in mice were recorded and the number of deaths within 14 d were recorded. The LD50 value was calculated according to Eq 1.

$$LD_{50} = l\, g^{-1}\{X_m - i\,(\Sigma p - 0.5)\} \quad \ldots\ldots\ldots (1)$$

where Xm is the logarithm of the maximum dose, p is the mortalities of KM mice in each group, i is the difference between the logarithm doses of two adjacent groups, and Σp is the sum of mortalities in each group.

Pharmacodynamic studies

Writhing test

Sixty healthy mice were randomly divided into 6 groups, which are SSTAM (12, 25, and 50 mg/kg) groups; SSTA (3 mg/kg) group; positive control group (aspirin [200 mg/kg]) and blank control group (0.5% sodium carboxymethyl cellulose). The gavage dose was 0.2 mL/10 g with continuous drug for 7 d. Mice were fasted for 12 h before the experiment. One hour after the last administration, mice were intraperitoneally injected with 0.6 % acetic acid (v/v, 10 mL/kg). The writhing times of mice within 20 min were recorded after administration. Inhibition rate (H, %) was calculated as in Eq 2.

$$H\,(\%) = (T_b - T_s)/T_b \times 100\%. \quad \ldots\ldots\ldots (2)$$

where T_b is the mean of writhing time in blank control group, and T_s is the mean of writhing time in administration group.

Hot plate test

Sixty healthy female mice with pain threshold values of 10 - 30 s were randomly divided into 6 groups as in the writhing test. The pain threshold values of mice (n = 3) were measured before administration, and the mean was calculated as the normal pain threshold value before administration. The mice received intragastric administration for 7 days. At 30, 60 and 120 min after the last administration, times that mice licked the hind foot on the hot plate were recorded. Times > 60 sec was recorded as 60 sec.

Ear swelling test

Mice were fasted for 12 h before the experiment. Sixty healthy female mice were randomly divided into 6 groups as in the writhing test. The mice received intragastric administration for 7 days and the gavage dose was 0.2 mL/10 g. One hour after the last administration, the front and back of the left ear were smeared with 0.05 mL of xylene; the right ear served as a blank control. After 1 h, mice were sacrificed, and two ears were cut along the auricle baseline. An 8-mm diameter punch was used to obtain a piece of the ear in the same location of the two ears, and immediately weighed. The difference between the left and right ears was the degree of swelling, and the inhibition rate (R, %) was calculated as in Eq 3.

$$R\,(\%) = \{(S_b - S_s)/S_b\}100 \quad \ldots\ldots\ldots (3)$$

where S_b is the mean degree of swelling in the blank control group, S_s is the mean degree of swelling in the administration group.

Cotton ball granulation test

Sixty mice were randomly divided into 6 groups as in the writhing test. After adaptive feeding for 1 week, 10 mg cotton balls (sterilized in an autoclave and dried in a vacuum drier at 55 °C) were set on the bilateral axillae of mice by subcutaneous implantation under sterile condition. Six hours later, intragastric administration was performed once a day for six consecutive days. Mice were sacrificed on the seventh day. The cotton balls were taken out carefully, dried in a vacuum drier at 55 °C to constant weight. The weight of the cotton ball before implantation was subtracted to determine the granuloma mass, and the inhibition rate (B, %) was calculated as in Eq 4.

$$B\,(\%) = \{(M_b - M_s)/M_b\}100 \quad \ldots\ldots\ldots (4)$$

where M_b is the mean of granuloma mass in blank control group, M_s is the mean of granuloma mass in administration group.

Hyperfunction test for abdominal capillary permeability

Sixty mice were randomly divided into 6 groups as in the writhing test. One hour after the last administration, 1 % Evans blue (0.1 mL/10 g) was injected into the caudal vein and 0.2 mL of 0.6 % glacial acetic acid was injected intraperitoneally. After 20 min, the mouse was sacrificed and the abdominal wall was washed thrice with 5 mL of normal saline. The washing solution was collected in 10 mL of EP and centrifuged at 2500 r/min for 5 min. The supernatant of the sample was measured with a UV-visible spectrophotometer at 590 nm, and the permeability of the abdominal capillary was evaluated based on the absorbance.

Statistical analysis

SPSS 16.0 statistical software (SPSS, Inc, Chicago, IL, USA) was employed to perform the statistical processing of the experimental data. Data were expressed as mean ± SD, and Mann-Whitney U test was employed to determine significance between two groups. $P < 0.05$ was considered statistically significant.

RESULTS

Pharmacokinetics of brucine and strychnine

Pharmacokinetics parameters of brucine and strychnine were exhibited in Table 1. After model fitting, it was shown that strychnine in mice conformed to a two-compartment model. The half-life of brucine in the low- and high-dose SSTAM groups were 9.321 h and 9.025 h, respectively, and 5.723 h in the SSTA group. The peak time in the low- and high-dose SSTAM groups were 2.167 and 2.333 h, respectively, and 0.5 h in the SSTA group. The peak concentration of the low- and high-dose SSTAM groups was 114.882 and 70.741 ng/L, respectively, and 102.358 ng/L in the SSTA group. The half-life of strychnine in the low- and high-dose SSTAM groups were 8.819 and 8.654 h, respectively, and 4.065 h in the SSTA group. The peak time in low- and high-dose SSTAM groups were 2.083 and 2.583 h, respectively, and 0.5 h in the SSTA group. The peak concentration in the low- and high-dose SSTAM groups was 102.546 and 66.621 ng/L, respectively, and 97.221 ng/L in the SSTA group (Table 1). These results indicated that SSTAM had a significantly slower release rate, longer peak time and more stable plasma concentration.

Toxicologic results for pre-experiment

Four mice died in the 400 mg/kg SSTAM group, while there were no deaths in the 50 and 100 mg/kg groups. 4 mice died in the 50 mg/kg SSTA group, while there were no deaths in the 5 and 15 mg/kg groups (Table 2). Combined with experimental observations, the main manifestations of poisoning were shortness of breath, low bend over, clonic convulsions and piloerection. Stiffness straight of two hind limbs and stiff tail were the symptoms of death.

Toxicologic results for full experiment

In the highest- and high-dose SSTA groups, toxicity symptoms and death occurred about 11 s after administration.

Table 1: Pharmacokinetic parameters of brucine and strychnine

Parameter	Unit	Brucine			Strychnine		
		SSTA	***Low-dose SSTAM***	***High-dose SSTAM***	***SSTA***	***Low-dose SSTAM***	***High-dose SSTAM***
$AUC_{(0-t)}$	ng/L·min	27710.058±6713.375	52765.441±4861.873	73229.074±11693.412	20505.849±2647.524	45665.431±5883.422	49871.817±7143.625
$AUC_{(0-\infty)}$	ng/L·min	29880.575±6933.055	55988.458±6100.245	86748.437±23475.132	25419.547±6350.216	49871.817±6911.270	66287.248±9024.682
$MRT_{(0-t)}$	H	6.456±1.8462	12.117±4.215	12.051±4.672	4.337±1.519	8.389±0.704	7.564±0.630
$MRT_{(0-\infty)}$	H	8.348±2.366	16.542±5.400	15.874±6.874	8.497±8.845	11.519±2.574	10.892±1.568
$t_{1/2}$	H	5.723±3.764	9.025±1.820	9.321±1.148	4.065±1.079	8.654±1.247	8.819±1.670
T_{max}	H	0.500±0.107	2.333±0.817	2.167±0.931	0.500±0.108	2.583±1.686	2.083±1.114
Zeta		0.003±0.002	0.061±0.011	0.058±0.018	0.067±0.010	0.074±0.0241	0.066±0.0305
Cz	ng/L	3.094±2.603	6.197±4.011	15.547±11.935	3.652±1.354	7.126±3.842	5.018±2.341
Cmax	ng/L	102.358±6.638	70.741±6.85	114.882±16.652	97.221±26.147	66.621±28.756	102.546±40.705

SSTAM, semen strychni total alkaloid microcapsules; SSTA, total alkaloid of semen strychni; AUC, area-under-curve; MRT, multiple-relaxation-time

Table 2: Number of deaths in mice in pre-test experiment (n = 4)

Group	Dose (mg/kg)	Number of deaths
SSTAM	50	0
	100	0
	200	1
	300	3
	400	4
SSTA	5	0
	15	0
	30	2
	40	3
	50	4

SSTAM, Semen strychni total alkaloid microcapsules; SSTA, total alkaloids of semen strychni

As the dose gradually decreased, the number of poisoned mice decreased, the poisoning time prolonged and the number of dead mice decreased (Table 3). In the highest dose SSTAM group, toxicity symptoms and death occurred approximately 20 sec after administration. As the dose gradually decreased, the latent period of toxic symptoms increased. Death occurred after the symptoms remained for approximately 30 sec. Some mice showed only mild toxicity, and the low-dose group did not show toxicity symptoms. Mice that were not dead within 1 day after administration were continuously observed for 14 days, and no deaths occurred. The LD50 value of SSTA was 30.27 mg/kg (95 % CI: 26.68 - 34.35 mg/kg). The LD50 value of SSTAM was 236.59 mg/kg (95 % CI: 157.62 - 355.14 mg/kg). The LD50 value of SSTAM was 2.23 times the SSTA, indicating that SSTAM could increase the dosage of administration and reduce the toxicity of strychni semen.

Analgesic effects

Writhing and hot plate tests were performed to evaluate analgesic effects. The pain inhibition in the 50 mg/kg SSTAM group was 50.2 %, which was statistically significant compared with the blank control group ($p < 0.01$). In addition, there was a significant difference between SSTA and SSTAM (25 and 50 mg/kg) groups ($p < 0.05$). The pain inhibition rate of the 12 mg/kg SSTAM group was 30.5 %, and there was no difference compared with the blank control group ($p > 0.05$), while the same result was found between the SSTA and blank control groups ($p > 0.05$). These results indicated that the high-dose SSTAM groups had an apparent analgesic effect to reduce twisting times, but low-dose SSTAM and SSTA groups had no analgesic effect because the low dose did not reach an effective concentration. As shown in Table 4, the pain threshold values of the SSTAM groups (25 and 50 mg/kg) were higher than blank control group ($p < 0.01$) and the SSTA group ($p < 0.01$) at 60 and 120 min. Compared with blank control group, the pain threshold value of the SSTA group was increased at 30 and 60 min ($p < 0.05$; Table 4). The results suggested that high-dose SSTAM had a higher pain threshold value, as well as a better analgesic effect.

DISCUSSION

By comparing the pharmacokinetic parameters of SSTAM and SSTA, we could find that through microencapsulation, the peak time and the release of brucine and strychnine were prolonged, and the *in vivo* stability of brucine and strychnine was improved. The LD50 value of SSTAM was 2.33-fold versus SSTA, indicating that SSTAM improved the safety dose. With the increase in dose, the effect was increased, thus showing a dose-effect relationship. SSTAM slowed the rate of drug release, reduced the peak of plasma concentration, and expanded the safe dose. Accordingly, SSTAM could increase the dosage of administration and reduce the toxicity of strychni semen.

Table 3: Number of deaths of mice (n = 10)

Group	Dose (mg/kg)	Dose logarithm	Number of deaths
SSTAM	15.52	1.91	0
	19.40	1.30	1
	24.40	1.38	2
	30.60	1.49	5
	38.40	1.60	7
	48.00	1.68	10
SSTA	110.10	2.04	0
	137.62	2.14	2
	196.60	2.30	3
	254.76	2.41	4
	307.20	2.49	7
	384.00	2.58	10

Table 4: Writhing and hot plate test results

Group	Dose (mg/kg)	Writhing test		Hot plate test			
		Writhing times	*Inhibition rate (%)*	Before administration	*After administration*		
					30 min	*60 min*	*120 min*
Blank control	-	53.2±8.6	-	18.5±2.4	22.4±2.6	19.3±3.2	21.3±3.1
Positive control	200	28.9±3.5*	45.7	17.6±3.1	30.0±3.1*	37.2±4.5*	24.8±3.8
SSTA	3	39.6±4.5	25.6	19.3±3.4	26.3±2.7*	24.4±2.5*	19.7±1.8
	12	37.0±4.0	30.5	17.5±4.8	20.2±1.6	27.6±1.0*	22.4±2.2
SSTAM	25	28.8±4.7$^{*\#}$	45.9	20.9±3.3	22.5±2.8	34.3±3.2$^{**\#}$	43.2±3.7$^{**\#}$
	50	26.5±4.4$^{**\#}$	50.2	21.1±2.7	26.4±1.6$^{*\#}$	38.2±1.1$^{**\#}$	44.5±3.2$^{**\#}$

SSTAM, semen strychni total alkaloid microcapsules; SSTA, total alkaloids of semen strychni. Compared with the blank control group; *p < 0.05, **p < 0.01, compared with total alkaloids group, #p < 0.05, ##p < 0.01

Anti-inflammatory effect

Table 5: Ear swelling, cotton ball granulation, and hyperfunction tests for abdominal capillary permeability among different groups

Group	Dose (mg/kg)	Ear swelling		Cotton ball granulation		Hyperfunction
		Swelling degree	Inhibition rate (%)	Granuloma mass	Inhibition rate (%)	Absorbance value
Blank control	-	13.0±3.2	-	19.1±4.6	-	0.7±0.1
Positive control	200	3.7±2.1**	71.5	8.0±2.8**	54.0	0.2±0.1**
SSTA	3	7.8±4.0*	39.8	12.1±4.3*	30.5	0.4±0.1*
	12	9.3±3.9*	28.3	10.0±2.9**	42.5	0.4±0.1**
SSTAM	25	4.2±2.6$^{**\#\#}$	68.0	9.6±2.5$^{**\#}$	44.5	0.3±0.1$^{**\#\#}$
	50	6.3±3.3$^{**\#}$	51.4	7.7±2.6$^{**\#}$	55.6	0.3±0.1$^{**\#\#}$

SSTAM, semen strychni total alkaloid microcapsules; SSTA, total alkaloids of semen strychni. Compared with the blank control group; *p < 0.05, **p < 0.01, compared with the total alkaloids group; #p < 0.05, ##p < 0.01

Recent studies have shown that brucine has a good anti-tumor effect and has an inhibitory effect. Brucine inhibits the transcription of human hypoxia-inducible factor (HIF)-1 gene and attenuates the expression of downstream genes of HIF-1, such as fibrin and matrix metalloproteinase-2 (MMP-2), thereby inhibiting HGP2 and smmc-7721 Cell transfer [2]. Brucine inhibits cell survival signaling transduction of key junction, and phosphorylation of protein kinase B [11]. Brucine induces apoptosis in human multiple myeloma (RPMI8226) cells by retarding the cell cycle and the destruction of mitochondrial membrane proteins and release of cytochrome C [4].

Strychnine is one of the toxic components of semen strychni and it is more toxic than brucine, but semen strychni has a non-negligible anti-tumor effect. MTT assay was used to assess the inhibitory effect of brucine and strychnine on human liver cancer cells (HepG2), and the results showed that strychnine also had a significant inhibitory effect on HepG2 cell proliferation [12]. Another study also confirmed that strychnine played a role in inhibiting tumor cell growth and anti-angiogenesis on the human breast cancer cell line, MCF-7, *in vitro* [13]. Therefore, pharmacokinetic studies on the SSTA of semen strychni and their preparations focused on the active pharmaceutical ingredients, such as brucine and strychnine.

The LC-MS/MS method has been intensively employed to investigate the pharmacokinetics of strychnine and brucine [14,15]. After a single oral administration of the SSTA from semen strychni at 4 dose levels, the C_{max} and AUC_{0-t} of strychnine and brucine increased and were proportional to the oral doses [14]. In present study, pharmacokinetics of strychnine and brucine in SSTAM and SSTA were studied by LC-MS/MS, and the Cmax, and AUC0−t of strychnine and brucine were also proportional to the dose. In a previous study, brucine-loaded stealth liposomes was prepared by the ammonium sulfate gradient method, and the stealth liposomes improved the pharmacokinetics of brucine [16]. The results showed that SSTAM had better pharmacokinetics than SSTA due to the slowed release rate, decreased peak concentration, longer peak time, and more stable

plasma concentration. With respect to acute toxicity, the LD_{50} value of SSTAM (236.59 mg/kg) was 2.23-fold versus the SSTA (30.27 mg/kg), indicating that SSTAM could effectively increase the dosage of administration and have less toxicity effect. The previous study found that LD_{50} value of brucine solution following intravenous injection was 13.17 mg/kg [16]. Compare with the crude drug, the LD_{50} value of processed products of semen strychni increased, and the toxicity lowered [17]. The acute toxicity of semen strychni showed a time-effect relationship in a dose-dependent manner [18], consistent with this study.

In this study, pharmacodynamics was evaluated by analgesic effects (writhing and hot plate tests) and anti-inflammatory effects (ear swelling, cotton ball granulation, and hyperfunction tests). Compound brucine prolonged the incubation period of pain caused by a hotplate in mice. A significant dose-effect relationship was noted in the analgesic study, and the apparent pharmacodynamic parameters were as follows: Ka = 0.09477/min; T1/2a = 7.3 min; Ke = 0.00203/min; T1/2e = 341.4 min; Tb = 1.3 min; Tmax = 41.4 min; Emax = 238.5; Te = 598.7 min; and Tc = 597.4 min [19]. The SSTA of the semen strychni transdermal patch improved the pain threshold caused by a hot board and reduced the duration of body writhing caused by acetic acid, showing a marked analgesic effect with high intensity of activity and long endurance time [20].

Semen strychni processed by cooled and heated milk could enhance the analgesic effect and the LD_{50} in mice, which illustrated the milk-impregnated processing technology could enhance active pharmaceutical effect and reduce the toxicity of strychni semen [21]. The results indicated that the pain inhibition of the high-dose SSTAM group was higher than the other groups and showed a significant analgesic effect to reduce twisting times. The pain threshold values of the high-dose SSTAM group was higher than the other groups. The high-, medium-, and low-dose SSTAM groups had anti-inflammatory effects, by the higher dose had better effects. These results indicated that the SSTAM group showed better analgesic and anti-inflammatory effects than the SSTA group.

CONCLUSION

The findings of this study indicate that SSTAM reduces the toxicity but enhances the efficiency of strychni semen in an animal model. The results also indicate that the analgesic and anti-inflammatory effects on SSTAM group are than that on SSTA group. Further studies in patients are to planned to ascertain the clinical applicability of the findings.

DECLARATIONS

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Conflict of interest

No conflict of interest is associated with this study.

Contribution of authors

We declare that this work was done by the author(s) named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors". Xinli Song, Daobin Yang conceived and designed the study, Xinli Song, Daobin Yang, Yunxia Wang and Wen Liu collected the data, Xinli Song, Yonglin Wang, Jiazhen Zhu analysed the data, Wen Liu, Yonglin Wang, Jiazhen Zhu wrote the manuscript. All authors read and approved the manuscript for publication in this journal.

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