Preparation of Bupleurum Nasal Spray and Evaluation on Its Safety and Efficacy

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Radix Bupleuri is widely used in traditional medicine for the treatment of fever, pain, and inflammation associated with influenza or the common cold. The essential oil extracted from the herb is generally claimed to play the major role in the efficacious treatment of fever. The purpose of the present study was to formulate an intranasal delivery system for the essential oil in an aqueous solution used in the form of nasal spray. From 450 g Radix Bupleuri was extracted the essential oil in the amount of about 0.2 ml, which was slightly water-soluble and viscous with low-fluidity. In order to dissolve the essential oil evenly in the aqueous solution, tween-80 (TW-80, used in 10% (w/v) solution), propylene glycol (PG) and diethylene glycol monoethyl ether (TC) were selected as the favorable solubilizing agents, whose amount was respectively determined by $L_{16}(4^5)$ orthogonal design. An aqueous solution with clarity and no ciliotoxicity was prepared when TW-80 8% (v/v), PG 14.4% (v/v) and TC 14.4% (v/v) were added. Employed to evaluate the acute toxicity, the rats grew well and were kept active and healthy within 14 d after an intranasal administration of this preparation at the dose of oil from 10 g Bupleuri/kg (50-fold higher than the clinical dose), indicating that there would be no serious toxicity at the normal dose. Intranasal administration of this preparation to 2 kg rabbits with fever induced by subcutaneous injection of turpentine decreased body temperature markedly (0.5, 0.8 and 1.0 °C respectively at the dose of oil from 1, 2 and 4 g Bupleuri/body). In addition, the administration significantly reduced fever in 200 g rats induced by intramuscular injection of colicine suspension (0.6 °C at the dose of oil from 0.8 g Bupleuri/body). The results suggest that the formulation of nasal spray for the essential oil from Radix Bupleuri can be potentially effective in the treatment of fever.

Key words Radix Bupleuri; nasal spray; antipyretic effect; nasal ciliotoxicity; essential oil

Radix Bupleuri is widely used in traditional medicine for the treatment of fever, pain, and inflammation associated with influenza or the common cold.[1—3] A number of *in vivo* studies have confirmed the antipyretic capacity of Radix Bupleuri in fevered animals. Oral administration of a Bupleurum decoction to rabbits with a heat-induced fever decreases body temperature to normal levels within 1.5 h.[3] Furthermore, the antipyretic activity has also been investigated in patients with fevers caused by the common cold, influenza, malaria and pneumonia. In one clinical study of 143 patients treated with the herb, fevers subsided within 24 h in 98.1% of all cases of influenza, and in 87.9% of all cases of the common cold.[3,4] The essential oil extracted from the herb is generally claimed to produce the antipyretic effects. Intraperitoneal injection of the essential oil isolated from Radix Bupleuri effectively decreased fever in mice induced by yeast injections.[5] For the treatment of fever, the essential oil has been extracted and formulated into a variety of dosage forms such as Bupleurum injection, major Bupleurum decoction and minor Bupleurum decoction. However, there exist deficiencies in the present medications. For example, the administration of Bupleurum injection, which can not be performed without the help of nurses, can cause serious myalgia, and Bupleurum decoction tastes considerably bitter, which make it hard for patients to accept them. The published results showed that Bupleurum injection given by intranasal administration had effectively reduced fevers in 97.0% of 100 cases.[6] However, the concentration of the essential oil in the Bupleurum injection is so low (oil from 1 g Bupleuri/ml) that 3—4 ml solution is needed one day, and the volume is too large for intranasal administration so that it has to be administered several times. Therefore, the purpose of this study was to formulate an intranasal delivery system, in which the concentration of the essential oil (oil from 15 g Bupleuri/ml) was much higher than that in the injection.

Nasal sprays are of practical use for their high-bioavailability, with easy-to-use and rapid-to-act features. Recently, dihydroergotamine, sumatriptan and zolmitriptan have been prepared and marketed in the form of nasal spray to treat migraine,[7—9] so have lyspressin, oxytocin and salcatonin for systemic use. Similarly, nimodipine, methotrexate and meptazinol have been attempted in our department, their results displaying a promising use in the treatment of diseases affecting the central nervous system (CNS).[10—12] Therefore, nasal spray was selected as the dosage form for the essential oil in our approach.

The essential oil extracted from Radix Bupleuri is slightly water-soluble and viscous with low-fluidity, so it can not be sprayed directly. Therefore, the crucial problem in our study was to find some favorable solubilizing agents to overcome the slight water solubility of the essential oil so as to optimize the preparation of Bupleurum nasal spray and investigate its nasal ciliotoxicity, acute toxicity and antipyretic capacity.

Experimental

Materials Both Radix Bupleuri and Bupleurum injection were supplied by Henan Nanyang Lixin Pharmaceutical Co., Ltd., China. As solubilizing agents, tween-80 (TW-80, Shanghai Dazhong Pharmaceutical Co., Ltd., China), propylene glycol (PG, Shanghai Ciji Pharmaceutical Co., Ltd., China) and diethylene glycol monoethyl ether (TC, Transcutol P®, Colorcon Corp., America) were used. Pineapple oil (Shanghai Peacock Flavor & Fragrance Co., Ltd., China) was used as the aromatizer. The strain of Esherichia

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coliform (*E. coli*, 922 style) was obtained from Shanghai Huashan Hospital affiliated to Fudan University, China. All other reagents were of the highest grade commercially available.

Extraction of the Essential Oil The essential oil was extracted from Radix Bupleuri by a modification of the steam distillation method reported by Wang B. J.[13] In brief, 450 g Radix Bupleuri was cut into short pieces to be dipped into 2700 ml saturated saline water for 12 h, and then the steam distillation step was performed, with 1350 ml distilled solution collected and saturated with NaCl. Subsequently, this solution was directly distilled again to collect 200 ml second-distilled solution, to which NaCl was added until it was saturated. At the end of the distillation step, 100 ml, 100 ml, 50 ml ethyl ether were applied in a sequent order to extract the essential oil from the second-distilled solution, and the collections were mixed with their remaining water eliminated with anhydrous Na_2SO_4. Finally, ethyl ether was removed under a strong air flow, thus gaining about 0.2 ml essential oil.

Screening of Solubilizing Agents The screening of solubilizing agents was performed by $L_{16}(4^5)$ orthogonal design.[14,15] As solubilizing agents, TW-80 (in 10% (w/v) solution), PG and TC were all examined at 4 levels. The essential oil, extracted from 4.5 kg Radix Bupleuri, was divided into 16 parts, and according to the $L_{16}(4^5)$ orthogonal table (Table 1), TW-80, PG and/or TC were added to each tube before water was filled until the final volume was 18.75 ml (1 ml solution containing oil from 15 g Bupleuri), followed by stirring for 5 min. Each experiment was done in triplicate.

The clarity (*CL*) and cost of each solution were chosen as the indexes to evaluate its quality. The solution's *CL* was estimated in 5 grades; the higher the grade, the clearer the solution. In addition, the parameter "cost integral" (*CI*), employed to express their cost, was calculated as follows:

$$CI = V_{\text{TW-80}} \times 0.8 + V_{\text{PG}} \times 1 + V_{\text{TC}} \times 5$$

$V_{\text{TW-80}}$, V_{PG} and V_{TC} stood for the volume of TW-80, PG and TC in each test, and their relative market prices were 0.8, 1 and 5, respectively, compared with that of PG. We intended to prepare a solution with high clarity and low cost, and as the former was considered as a major evaluating mark, the weighting indexes of *CL* and *CI* were respectively fixed on 0.7 and 0.3. The integrated mark (*IM*) of each solution was calculated as follows:

$$IM = \left(\frac{CL}{CL_{\max}}\right) \times 0.7 \times 100 + \left(\frac{CI_{\max}}{CI_{\max} + CI}\right) \times 0.3 \times 100$$

$CL_{\max}$ and $CI_{\max}$ respectively referred to the maximum value of *CL* and *CI*. The values of *IM* were analyzed by intuitionistic analysis (INAN), followed by analysis of variance (ANOVA).

Preparation of Bupleurum Nasal Spray In the study the solubilizing agents were screened for the best formulation selected to prepare Bupleurum nasal spray. The mixture of TW-80, PG and TC was mingled with the essential oil extracted from 450 g Radix Bupleuri, followed by stirring for 5 min. Afterwards, benzyl alcohol 0.15% (v/v), $Na_2S_2O_5$ 0.1% (w/v), pineapple oil 1% (v/v) were added in a sequent order to act as preservative, antioxidant and aromatizer, which was stirred again for 2 min before water was added until the final volume reached 30 ml. Finally, 10% (w/v) NaOH was employed to adjust pH to 4.5.

Nasal Ciliotoxicity Nasal ciliotoxicity studies were carried out using both *in situ* toad palate model and *in vivo* rat nasal mucosa model.[16] In the toad experiment, the upper palate of toad (30—40 g, ♂♀, Experimental Animal Center of Fudan University, China) was exposed and treated with 0.5 ml test Bupleurum nasal spray (oil from 15 g Bupleuri/ml) for 1 h, and then rinsed quickly with physiological saline. Finally, the palate was dissected and the mucocilia was immediately examined with an optical microscope (Buffald, America). Sprague–Dawley rat (180—220 g, ♂♀, Experimental Animal Center of Fudan University, China) was also employed in this study. For the intranasal administration, 60 μl Bupleurum nasal spray (oil from 15 g Bupleuri/ml) was administered *via* a polyethylene 10 (PE 10) tube attached to a microlitre syringe inserted 1 cm into the rats' nostrils once a day, and the administration lasted for 7 d. The rats were sacrificed 24 h after the last administration, with the nasal mucosa peeled off, and the mucocilia was examined under a scanning electronic microscope (S-520, Hitachi, Japan). Physiological saline and sodium deoxycholate (one of serious nasal ciliotoxicity agents, 1% (w/v) solution) were used as the negative and positive control, respectively. In the experiments the bases (the solution containing only the excipient) was also examined.

Acute Toxicity The acute toxicity was evaluated in Sprague–Dawley rats (250—300 g, ♂♀, Shanghai SLAC Laboratory Animal Co. Ltd., China). Bupleurum nasal spray (oil from 15 g Bupleuri/ml) was administered to the rats at the dose of oil from 10 g Bupleuri/kg (50 μl solution given every 5 min in the way described above), and then their physiological characters such as daily diet and behavior were observed and recorded after the last administration, which was performed once a day for 14 d. If one rat had died during the experiments, it would have been dissected at once for pathologic examination to identify the cause of its death. And the bases and normal saline were used as the excipient and blank control, respectively. The data were analyzed by Student's *t*-test with the Stata 7.0 program.

Antipyretic Effect on Rabbits The New Zealand-derived white rabbits (2.0±0.2 kg, ♂, Shanghai Experimental Animal Center, Chinese Academy of Sciences) were used in this study. Firstly, pyrexia was induced in rabbits with a subcutaneous injection of 0.2 ml/kg turpentine. Then their temperatures were taken 16 h after the injection to determine the rabbits' pyretic responses. Finally, the rabbits whose temperatures had risen over 1 °C were selected and divided into six groups randomly for the antipyretic test. After that, their temperatures were taken 1 h prior to drug administration and served as pre-drug control. The solution used for intranasal administration was bottled in 5 ml nasal spray bottle (Pfeiffer Corp., Germany), which consisted of 5 ml brown bottle and a quantitative spray pump (each spray is 0.1 ml). The first group (the negative control) and the second group (the excipient control) received 0.2 ml/body physiological saline and 0.2 ml/body bases intranasally, respectively. The test groups were ranked the third, fourth and fifth, which received 0.1, 0.2 and 0.4 ml/body Bupleurum nasal spray (oil from 10 g Bupleuri/ml, the original nasal solution was diluted with the bases.), respectively. The sixth group was treated as the positive control, and received intramuscularly 2 ml/body Bupleurum injection (oil from 1 g Bupleuri/ml), which has been affirmed to have obvious antipyretic effect on patients.[17] And their temperatures were recorded at predetermined time intervals after the administration.

Antipyretic Effect on Rats The antipyretic effect was also evaluated in rats. The colicine produced from *E. coli*. prior to the antipyretic tests was used as the pyrogen. Firstly, 2 ml *E. coli*. suspension (1 ml solution containing 1×10^9 spores) was added to 50 ml nutrient broth (Shanghai Municipal Center for Disease & Prevention, China, 1.8% (w/v) in solution) for incubation at 30 °C with gentle to-and-fro vibrations at 90 rpm for 10 d. At the end of the incubation step, the culture medium was centrifuged 8000 rpm for 8 min at 5 °C. The precipitate was collected and dissolved in 1 ml glycerol by grinding. Then both the supernatant and precipitate-glycerol suspension were sterilized at 121 °C for 15 min. Finally, the pyrogen was obtained when the precipitate-glycerol suspension was diluted to 10 ml with the supernatant, and stored at 4 °C until used.

Sprague–Dawley rats (200±10 g, ♂♀, Shanghai SLAC Laboratory Animal Co. Ltd., China) were fevered with colicine at the dose of 2 ml/kg by an intramuscular injection. Five hours later their temperatures were taken to determine their pyretic responses. Then those whose temperatures had risen more than 1 °C were selected and divided into six groups randomly for the antipyretic test. The pre-drug control temperatures were taken 1 h prior to the drug administration. For the intranasal administration, the drug solution was given *via* a PE 10 tube attached to a microlitre syringe inserted 1 cm into the rats' nostrils. The first group (the negative control) and the second group (the excipient control) received 40 μl/body physiological saline and 40 μl/body bases intranasally, respectively. The third, fourth and fifth group were given 20, 40 and 80 μl/body Bupleurum nasal spray (oil from 10 g Bupleuri/ml, the original nasal solution was diluted with the bases), respectively. The sixth group treated as the positive control was injected intramuscularly 0.4 ml/body Bupleurum (oil from 1 g Bupleuri/ml). And the temperatures were recorded at predetermined time intervals after administration. All of the antipyretic tests' results were analyzed statistically through Student's *t*-test with the Stata 7.0 Program.

Results and Discussion

Preparation of Bupleurum Nasal Spray We firstly investigated how the amount of the solubilizing agents including TW-80, PG and TC affected the clarity of the aqueous solution containing the essential oil. According to the ANOVA results (Table 2), TW-80, PG and TC presented marked and dose-dependent solubilizing effects, and their respective order of capacity was TC>PG>TW-80. And INAN results (I_j, II_j, III_j and IV_j) (Table 1) indicated that if the combination of $A_2B_4C_4$ (1.5 ml TW-80, 2.7 ml PG and 2.7 ml TC con-

Table 1. $L_{16}(4^5)$ Orthogonal Design Experimental Table[a)]

Test No.	Column No.[b)]					The indexes of evaluation[c)]		
	1 (A)	2 (B)	3 (C)	4 (D)	5 (E)	*CL*	*CI*	*IM*
1	1 (0.90)	1 (0.90)	1 (0.00)	1	1	14.00	27.38	41.38
2	1 (0.90)	2 (1.50)	2 (0.90)	2	2	28.00	21.47	49.47
3	1 (0.90)	3 (2.10)	3 (1.80)	3	3	42.00	17.66	59.66
4	1 (0.90)	4 (2.70)	4 (2.70)	4	4	70.00	15.00	85.00
5	2 (1.50)	1 (0.90)	2 (0.90)	3	4	28.00	21.58	49.58
6	2 (1.50)	2 (1.50)	1 (0.00)	4	3	28.00	25.87	53.87
7	2 (1.50)	3 (2.10)	4 (2.70)	1	2	70.00	15.05	85.05
8	2 (1.50)	4 (2.70)	3 (1.80)	2	1	70.00	17.02	87.02
9	3 (2.10)	1 (0.90)	3 (1.80)	4	2	42.00	17.81	59.81
10	3 (2.10)	2 (1.50)	4 (2.70)	3	1	70.00	15.11	85.11
11	3 (2.10)	3 (2.10)	1 (0.00)	2	4	28.00	24.52	52.52
12	3 (2.10)	4 (2.70)	2 (0.90)	1	3	42.00	19.67	61.67
13	4 (2.70)	1 (0.90)	4 (2.70)	2	3	56.00	15.16	71.16
14	4 (2.70)	2 (1.50)	3 (1.80)	1	4	56.00	17.16	73.16
15	4 (2.70)	3 (2.10)	2 (0.90)	4	1	56.00	19.77	75.77
16	4 (2.70)	4 (2.70)	1 (0.00)	3	2	56.00	23.31	79.31
I_j[d)]	235.51	221.93	227.08					
II_j	275.53	261.61	236.49					
III_j	259.11	273	279.65					
IV_j	299.39	313	326.32					

a) There were 3 variables and 4 levels studied in the experiment. *b*) Variable A, B and C referred to TW-80, PG and TC, respectively, and the volume (ml) of them used in each test was shown in the table. And variable D and E stood for the error during the test. *c*) *CL*, *CI* and *IM* referred to clarity, cost integral and integrated marks, respectively. *d*) *j* represented variable A, B and C. And I_j, II_j, III_j and IV_j meaned the sum of *IM* in each level of variable *j*.

Table 2. ANOVA Results for the Screening of Solubilizing Agents

The difference source	Variable A	Variable B	Variable C	Error
Sum of squares	543.79	1052.95	1550.75	297.32
Degrees of freedom	3	3	3	6
Mean variance	181.26	350.98	516.92	49.55
F	3.66	7.08	10.43	
p	$0.05<p<0.1$	$0.01<p<0.05$	$p<0.01$	

$F_{0.10}(3,6)=3.29$, $F_{0.05}(3,6)=4.76$, $F_{0.01}(3,6)=9.78$

tained in 18.75 ml solution) or of $A_4B_4C_4$ (2.7 ml TW-80, 2.7 ml PG and 2.7 ml TC contained) was adopted, the value of *IM* would be better than other combinations. Therefore, $A_2B_4C_4$ was selected as the last combination due to its lower-cost. From 450 g Radix Bupleuri, three batches of essential oil were extracted respectively and dissolved in 30 ml aqueous solution containing 8% (v/v) TW-80, 14.4% (v/v) PG and 14.4% (v/v) TC. As a result, they were prepared with clarity.

On the one hand, the essential oil contained γ-lactone which was more stable in acid solution. On the other hand, the proper pH of the drug solution for the nasal administration was 4.0—7.0 to avoid nasal irritation. Therefore, pH in the preparation was adjusted to 4.5 with NaOH.

In our approach, saturated saline was used in distillation so as to increase the extracting efficiency of the essential oil thanks to the advantages as follows: Firstly, the essential oil that exists in the oil cell was easier to transfer to saturated saline than to water because of the function of dialysis; secondly, its solubility in saturated saline was lower than that in water so that there could exist more free oil in saline; thirdly, the boiling point of saturated saline was higher than that of water, so that saline was better suited for the distillation of the high-boiling constituents of the essential oil. The results showed that the absorbency of the distilled solution at 278 nm was greater than that obtained by the traditional method, which was consistent with the published reports.

Bupleurum injection contains a single solubilizing agent, TW-80. However, Bupleurum nasal spray holds a variety of solubilizing agents, because the concentration of the essential oil in the former is 15-fold lower than in the later. In the previously reported studies, PG was prevalently applied for its ideal solubilizing capacity, high biocompatibility and low toxicity to solubilizing the slightly water-soluble drugs.[18—20] In addition, it was testified that TC could be used as a permeation enhancer to increase the drug's transport,[21,22] or utilized as a solubilizing agent.[23] Therefore, in addition to TW-80, PG and TC were employed as solubilizing agents in our study.

Nasal Ciliotoxicity Nasal mucociliary clearance plays a crucial role in protecting the respiratory system from damage by inhaled substances.[24] Therefore, it is vital to examine the influences of drugs and drug excipients on nasal mucociliary clearance before clinical application. In our study, optical microscopic results showed that there were a great number of cilia at a fast beating rate on the edge of the mucosa treated with the bases or Bupleurum nasal spray for 1 h (Fig. 1), and the beating lasted for about 12 h after the palate was dissected, indicating that both the essential oil and excipient showed no obvious effect on the cilia movement. The scanning electron microscopic results displayed that the cilia were bushy and intact on the surface of the mucosa after the continuous administration of the bases or Bupleurum nasal spray for 7 d (Fig. 2), indicating that they also presented no marked effect on the length and density of the cilia. In addition, hyperaemia, tumefaction and ulcer were all not observed on the surface of the nasal mucosa by macrography. Based on the above results, we can conclude that both the essential oil and excipient possess no serious nasal ciliotoxicity

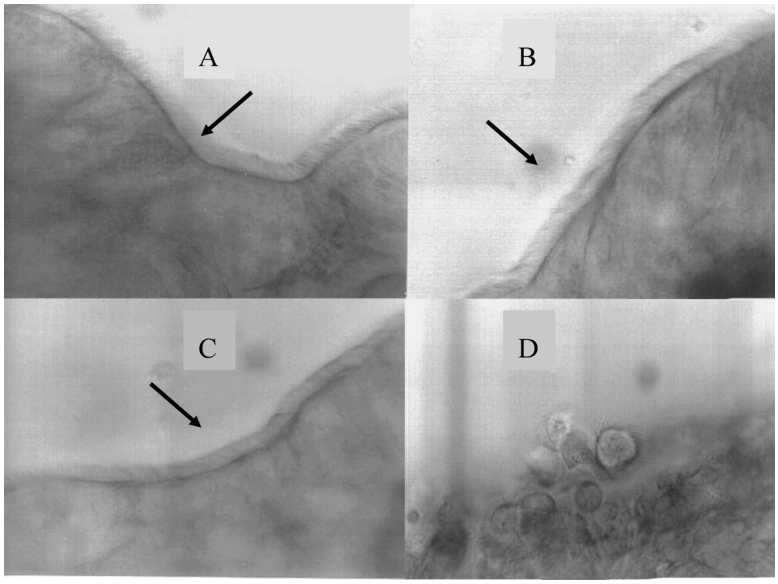


Fig. 1. Optical Microscopic Images of (A) Normal Saline (the Mucocilia Was Intact and Beating Actively, $n=6$), (B) the Bases and (C) Bupleurum Nasal Spray (the Phenomenon of Them Were the Same as That of Normal Saline, $n=6$), (D) 1% Sodium Deoxycholate Solution (the Mucocilia Could Not Be Observed, $n=6$)

The cilia on the mucosa are indicated by arrows.

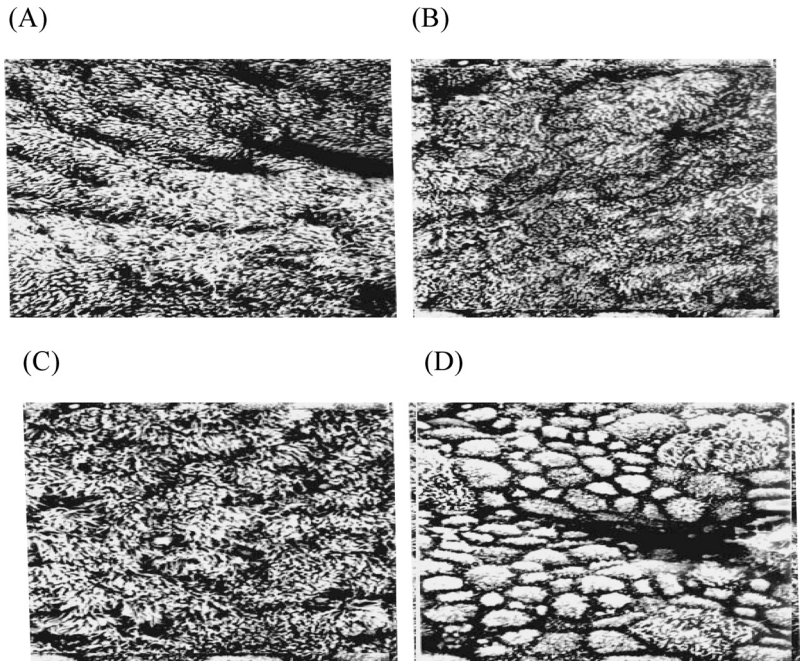


Fig. 2. Scanning Electron Microscopic Pictures of (A) Normal Saline (the Mucocilia Was Dense and Intact, and Arranged Trimly, $n=4$), (B) the Bases and (C) Bupleurum Nasal Spray (the Phenomenon of Them Were the Same as That of Normal Saline, $n=4$), (D) 1% Sodium Deoxycholate Solution (There Was Few Cilia Observed on the Surface of the Mucosa, $n=4$)

and irritability.

Acute Toxicity Both drugs and their excipient could produce some adverse effects on patients if used at an excessive dose. Therefore, it is imperative that the effective and safe doses of the drugs be prescribed before clinical research. In our approach, the acute toxicity investigation was conducted in the rats in an attempt to determine the drug's median lethal dose (LD_{50}) and evaluate its toxicity, with the results indicating that all of the rats, treated with oil from 10 g Bupleuri/kg (the dose was above 50-fold higher than the clinical dose), remained active and free of cough, asthma, vomit and asphyxia during the whole experiments. The test group showed a worse appetite than the controls at the first day after drug administration, but their appetites began to restore at the second day. And there were no obvious differences in weight between the test group and either of the control groups from beginning to end (Fig. 3), indicating that the spray generated no serious effect on the growth of the rats. Consequently, it could be affirmed that Bupleurum nasal spray at the dose of oil from 10 g Bupleuri/kg showed no marked effect on the rats' physiological characters, and we could conclude that the LD_{50} of Bupleurum nasal spray was

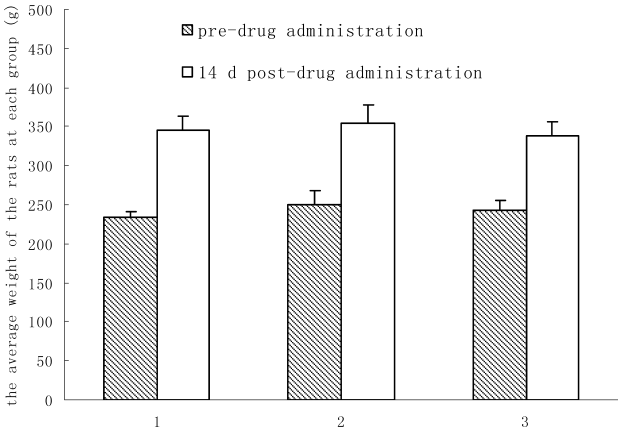
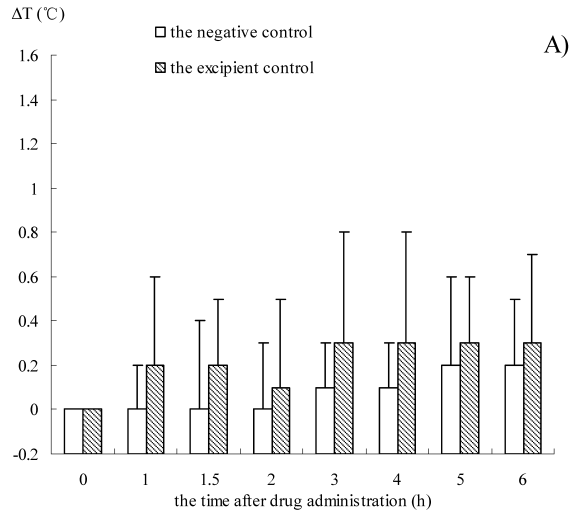


Fig. 3. Effect of the Drug Solutions on the Weight of the Rats

1, the negative control group, 2, the excipient control group, 3, the test group.



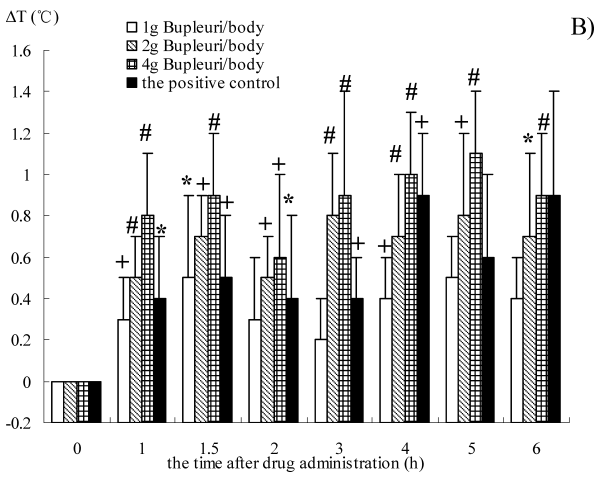
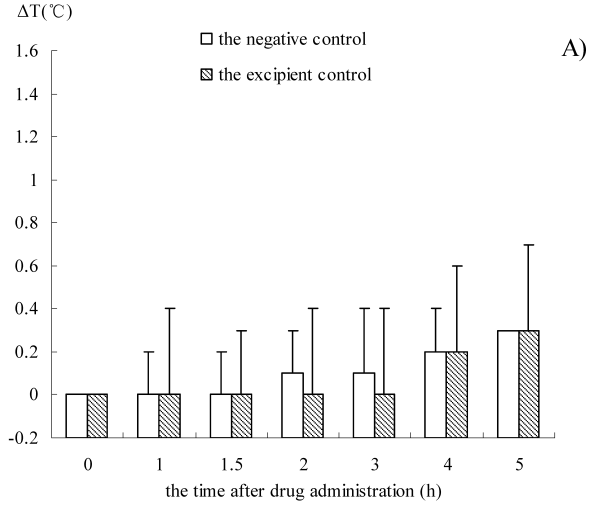


Fig. 4. Effect of Drug Solutions on the Pyrexia Induced by Turpentine in Rabbits

(A) The negative and excipient control groups (n=8), (B) the test-drug groups and the positive control group (n=10). $*0.05<p<0.10$, $+0.01<p<0.05$, $\#p<0.01$ *vs.* the negative control, Student's t-test. ΔT standed for the body temperature difference between pre-drug administration and post-drug administration, and it was expressed by mean±S.D.



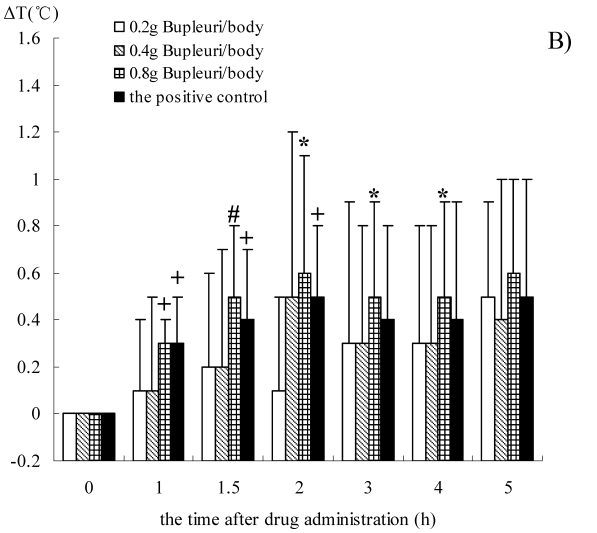


Fig. 5. Effect of Drug Solutions on the Pyrexia Induced by Colicine in Rats

(A) the negative and excipient control groups (n=10), (B) the test-drug groups and the positive control group (n=10). $*0.05<p<0.10$, $+0.01<p<0.05$, $\#p<0.01$ *vs.* the negative control, Student's t-test. ΔT standed for the body temperature difference between pre-drug administration and post-drug administration, and it was expressed by mean±S.D.

above oil from 10 g Bupleuri/kg for rats.

Antipyretic Effect In order to determine the drugs' antipyretic effect, two kinds of fevered animals (one induced by chemical and the other by microbe) are needed, with the body temperature differences between fevered and normal animals at 1 °C or above. In this study, pyrexia was induced in rabbits by turpentine, which was easily available and capable of stabilizing the fevered model for more than 8 h. Meanwhile, pyrexia was induced by colicine, rather than yeast's suspension,[25,26] in rats, because the preliminary results displayed that the fevers induced by colicine could avoid the great individual diversity found in the yeast test. In the tests on the rabbits' fevers (Fig. 4), the maximum of ΔT (the average body temperature differences between pre-drug administration and post-drug administration) was 0.5, 0.8 and 1.0 °C, and the antipyretic capacity lasted for 4, 6 and 6 h, respectively at the doses of oil from 1, 2 and 4 g Bupleuri/body, and in the positive control group, the data were 0.5 °C and 4 h, re-

spectively. In the tests on the rats' fevers (Fig. 5), Bupleurum nasal spray at the doses of oil from 0.2 and 0.4 g Bupleuri/body did not exhibit obvious antipyretic effect. However, when the dose increased to oil from 0.8 g Bupleuri/body, the maximum of ΔT was 0.6 °C, and the antipyretic capacity lasted for 4 h, and in the positive control group, the data were 0.5 °C and 2 h, respectively. These results showed that Bupleurum nasal spray exhibited dose-dependent and significant antipyretic capacity in rabbits at all the doses used above and in rats at the dose of oil from 0.8 g Bupleuri/body, and the effects on fevered rabbits were much greater than those produced with Bupleurum injection.

The spray produced obvious antipyretic effect on the rabbits at all the doses, but on the rats only at the high dose, the reason for which was supposed that the spray was administered to the rabbits by nasal spray pump, with the drug solution spurted in mist, and to the rats by microliter syringe, with the solution streamed in drop, so the former was more likely to be absorbed than the later. When the spray is given to the patients, the nasal spray pump is employed, thus the antipyretic effect on them being more similar to the rabbits. Therefore, we can forecast that the spray would produce obvious antipyretic effect on patients with a fever at the dose of oil from 9—12 g Bupleuri/body (0.6—0.8 ml solution a day), based on rabbit to human dosage conversion formula[27] showed below:

$$\text{human equivalent dose} = \text{rabbit dose in g/kg} \times (\text{rabbit weight in kg/human weight in kg})^{0.33}$$

Conclusion In amount, 30 ml Bupleurum nasal spray with clarity can be prepared by mixing the essential oil, extracted from 450 g Radix Bupleuri, with TW-80 8% (v/v), PG 14.4% (v/v) and TC 14.4% (v/v). And both essential oil and excipient produced no serious nasal ciliotoxicity, irritability and acute toxicity. Moreover, Bupleurum nasal spray exhibited dose-dependent and significant antipyretic capacity on both fevered rabbits and rats, and the effects on the fevered rabbits were much greater than those produced by Bupleurum injection. Therefore, we can conclude that Bupleurum nasal spray is safe for intranasal administration and potentially efficacious against fever clinically, especially among children.

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