Original Article

Hypotensive response in rats and toxicological mechanisms induced by shuanghuanglian, an herbal extract mixture

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ABSTRACT: Shuanghuanglian (SHL), an extract mixture isolated from three medicinal herbs, has been used in China as an injection in traditional Chinese medicine to treat viral or bacterial infection. This study examined the hypotensive response in rats induced by SHL and its possible mechanisms. Mean arterial pressure (MAP) and electrocardiograms (ECGs) were studied after intravenous injection of histamine and SHL. Diphenhydramine, an H_1 receptor antagonist, the compound 48/80, a promoter of histamine release, and cromolyn, a histamine release inhibitor, were also used to investigate the potential mechanisms of that response. In addition, the histamine level in plasma was measured after administration of SHL and compound 48/80. Both SHL and histamine led to a MAP reduction immediately but did not affect ECGs when initially administered in a similar manner, though this reduction was partially attenuated by diphenhydramine. Pretreatment blocked the rats' reaction to compound 48/80 but not to SHL. The plasma histamine level in rats was also elevated by SHL. SHL can induce severe hypotension through histamine release upon initial administration. In combination with the direct effects of its histamine-like substances on target tissue, SHL likely has the potential to cause an anaphylactoid reaction.

Keywords: Herbal extract mixture, shuanghuanglian, mean arterial pressure, anaphylactoid reaction, histamine

1. Introduction

Shuanghuanglian (SHL) is an extract mixture isolated from three medicinal herbs: Radix scutellariae (root of *Scutellaria baicalensis Georgi*), Flos lonicerae japonicae (flower of *Lonicera japonica* Thunb.) and Fructus forsythiae (fruit of *Forsythia suspensa* Thunb. Vahl). SHL has been used for over 40 years as an injection in traditional Chinese medicine to treat viral or bacterial infection. This medicine is commercially available and widely used to treat upper respiratory infections, pharyngitis, acute bronchitis, tonsillitis, and pneumonia in China because of its strong antiviral and bacteriostatic activity and ability to enhance immune function. However, adverse effects have frequently been reported with the extensive clinical use of SHL (*1*), and a patient who used SHL died, according to the State Food and Drug Administration (SFDA), in February and September 2009 (*2*).

Nearly 10% of hospitalized patients and over 7% of the general population suffer adverse drug reactions, including hypersensitivity reactions (HSRs) (*3*). HSRs fall into two categories, anaphylaxis and anaphylactoid reactions (*4,5*). Anaphylaxis is a specific immunoglobulin IgE-mediated antigen-induced reaction to various allergens resulting in the release of a mediator such as histamine, tryptase, or leukotrienes (*6*). The incidence of anaphylaxis is unknown but is estimated to be 1/6,000-1/20,000, or 1/10,000 of the general population (*5*). Anaphylactoid reactions have an identical clinical presentation but involve mediators besides IgE and are caused by various mechanisms (*7*). Recent estimates suggest that these non-IgE-mediated anaphylactoid reactions may account for as much as 77% of all immune-mediated hypersensitivity reactions (*8*). The occurrence of HSRs induced by SHL is very high (*1*), which may be related to anaphylactoid reactions. Mediator measurement combined with symptom monitoring is the primary means of studying anaphylactoid reaction. Sitter *et al.* (*9*) sought to investigate anaphylactoid reactions by detecting the plasma histamine level, heart rate, mean arterial pressure (MAP), and skin reaction in humans and dogs (*4*), and they indicated that the plasma histamine level and MAP may be sensitive indices. Histamine release from mast cells, stimulated by the compound 48/80, for instance, is considered to

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be a cause of anaphylactoid reactions. Cromolyn has also been shown to stabilize mast cells and prevent histamine release (*10*). Hypotension, which often occurs with an anaphylactoid reaction, can be induced by histamine or histamine-like substances that are usually blocked by histamine receptor antagonists (*e.g.* diphenhydramine) (*9*). The current study sought to examine changes in the plasma histamine level and MAP after intravenous administration of SHL and histamine in rats in order to determine the possible cause of HSRs induced by SHL and their correlation with anaphylactoid reactions.

2. Materials and Methods

2.1. *Animals*

Male Wistar rats (Center for New Drugs Evaluation of Shandong University) used in tests weighed 250-350 g, were housed for 7 days at a room temperature of 23 ± 1°C, and were given free access to food and water. Each group tested consisted of 5 rats. All experiments were performed in accordance with the Care and Use of Laboratory Animals of the US Department of Health and Human Services. The study protocol was approved by the local "Animal Ethics Committee".

2.2. *Drugs and Chemicals*

SHL as is officially registered in the Chinese Pharmacopoeia (CP) was provided by the Second Traditional Chinese Medicine Factory of the Harbin Pharmaceutical Group (Harbin, China). According to the CP, the quality controls for SHL (freeze-dried powder, mg/600 mg) are baicalin 128-173, chlorogenic acid 8.5-11.5, and forsythin 1.4-2.1, respectively (Figure 1) (*11*). SHL was required to have a consistent fingerprint (*12*). Compound 48/80, histamine dihydrochloride, diphenhydramine hydrochloride, and cromolyn were purchased from Sigma (St. Louis, MO, USA). A histamine ELISA kit was purchased from Rapid Bio Lab (Calabasas, CA, USA).

2.3. *Effects of histamine, compound 48/80, SHL, diphenhydramine, and cromolyn on MAP*

Rats were anesthetized with sodium pentobarbital. After a trachea tube was inserted, a carotid artery was isolated and catheterized with an Angio-set IV catheter system for MAP measurement (Biopac MP System). MAP was recorded every 10 sec (*13,14*). In a separate study, blood samples used for determining plasma histamine levels were collected from the carotid artery (*15*).

The jugular vein was isolated and used for intravenous administration. In experiments involving multiple doses or involving both an injection and an infusion, both jugular veins were exposed prior to the initiation of the experiment and used alternatively. All bolus injections were delivered in 30 sec in a volume of 0.5 mL. The infusion rate used for cromolyn was 0.5 mL/min (*13,15*). A saline vehicle was employed for all compounds, and concentrations of dosing solution were dependent upon the concentration required to deliver 0.5 mL injections and a 0.5 mL/kg/min dose for infusions.

To determine the effect of SHL or histamine on MAP, 100 to 400 mg/kg of SHL (according to the CP, the adult dose of SHL for clinical use is 60 mg/kg) (*11*) or 0.1 to 5.0 mg/kg of histamine (*13*) was given *via* the jugular vein (n = 5). Saline served as the vehicle for the control group.

To investigate the effects of repetitive treatments, 1.0 mg/kg of histamine (n = 5) or 200 mg/kg of SHL (n = 5) in saline was given by bolus injection *via* the jugular vein. If the MAP returned to the baseline, a second treatment with the same dose was given 15 min later. The maximum reduction in MAP and duration of hypotension were used to compare the responses induced by the two treatments.

To determine the effects of diphenhydramine on the response induced by histamine and SHL, 0.1 mg/kg of histamine, or 200 mg/kg of SHL was given *i.v.* by bolus injection. Fifteen minutes later, diphenhydramine (4.6 mg/kg) was injected as a bolus. Fifteen minutes later, histamine or SHL was given again in the same dose as before (n = 5).

To determine the effects of cromolyn on the response induced by compound 48/80 and SHL, animals were infused with saline (0.1 mL/min) or cromolyn (4 mg/kg/min) for 5 min and then treated with 0.1 mg/kg of compound 48/80 or 200 mg/kg of SHL *i.v.* (bolus) immediately after saline or cromolyn infusion (n = 5) (*13,15*).

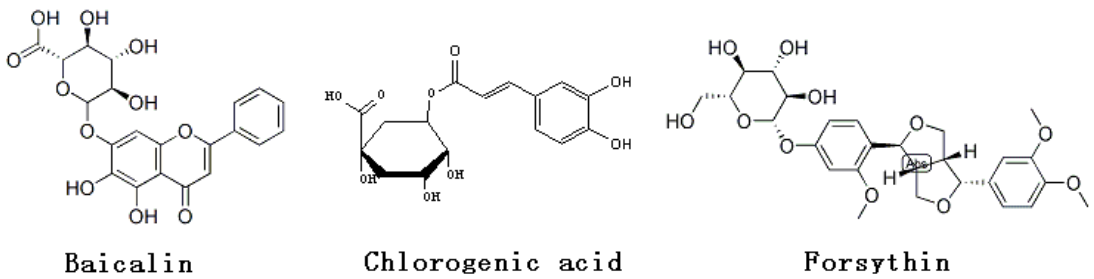

Figure 1. Natural product structures of major components in SHL. Quality controls for SHL (freeze-dried powder, mg/600 mg) were baicalin 128-173, chlorogenic acid 8.5-11.5, and forsythin 1.4-2.1, respectively.

2.4. *Histamine release*

In a separate experiment to determine the effects on histamine release, animals (n = 5) were treated with saline, compound 48/80 (0.1 mg/kg), or SHL (400 mg/kg) as described above. Blood samples (1 mL) were collected from the carotid artery into EDTA vacutainers 5 min after the treatment. Plasma samples were stored at -20°C until assay (*15*). Histamine concentration was measured using an ELISA kit.

2.5. *Statistical analysis*

All results are expressed as mean ± S.E.M. Experimental groups were compared using a Student's paired t test, with $p < 0.05$ considered to indicate a significant difference.

3. Results

3.1. *Responses to histamine, SHL, diphenhydramine, compound 48/80, and cromolyn*

Intravenous bolus injection of SHL resulted in a decrease in MAP in the dose range of 100 to 400 mg/kg in anesthetized rats but did not influence ECGs (data not shown); this decrease was not dose-dependent (Figure 2A). The decrease in MAP was observed immediately after the initiation of injection, and MAP returned to its baseline level about 2 to 3 min later. To investigate the hypotensive response induced by SHL, the effects of histamine on MAP were determined. Following bolus injection of 0.1 mg/kg of histamine *i.v.*, MAP decreased approximately 50% and then returned to its pretreatment level within 2 min (Figure 2B). Increasing doses of histamine from 0.1 to 1.0 mg/kg prolonged the duration of action but not the maximum effect. At a dose of 5 mg/kg, the MAP decreased more sharply and did not return to its normal level until the end of monitoring.

When SHL was given twice, 15 min apart, to the same rat in a dose of 200 mg/kg *i.v.* (bolus), the hypotensive response was similar (Figure 3A). Repetitive dosing with 1.0 mg/kg of histamine did not alter the maximum effect and hypotensive duration, resembling the effects of SHL (Figure 3B).

To investigate the effect of diphenhydramine on the hypotensive response induced by histamine, animals were treated with 1.0 mg/kg of histamine, 4.6 mg/kg of diphenhydramine, and 1.0 mg/kg of histamine in turn in intervals of 15 min. The hypotensive response induced by the second injection of histamine was found to be much weaker than the first, as the changes in MAP from its baseline to nadir were 69.1 ± 9.6 mmHg and 15.3 ± 2.8 mmHg, respectively ($p < 0.0004$) (Figure 4A). This demonstrates that diphenhydramine partially blocked the hypotensive response induced by histamine. When rats were given 200 mg/kg of SHL, 4.6 mg/kg of diphenhydramine, and 200 mg/kg of SHL in turn in intervals of 15 min, the hypotensive response induced by the second treatment of SHL was partially blocked by diphenhydramine, with a change in MAP of 38.1 ± 4.6 mmHg, compared to 60.3 ± 14.3 mmHg for the first treatment with SHL ($p < 0.05$) (Figure 4B).

To assess the effect of cromolyn on hypotension induced by the compound 48/80 in this model, saline or cromolyn was given to rats prior to the injection of the compound 48/80 (Figure 5A). When saline was infused (0.1 mL/kg/min, 5 min) prior to the compound 48/80, MAP decreased significantly from 162.8 ± 12.4 mmHg to the nadir of 104.5 ± 12.0 mmHg ($p < 0.003$). When the compound 48/80 was used after the infusion of cromolyn, the slight change in MAP from its baseline to 16 min demonstrated that cromolyn significantly inhibited the response of the compound 48/80. This demonstrates that cromolyn significantly attenuated the hypotension induced by the compound 48/80 (change in MAP from its baseline to 16 min, saline *vs.*

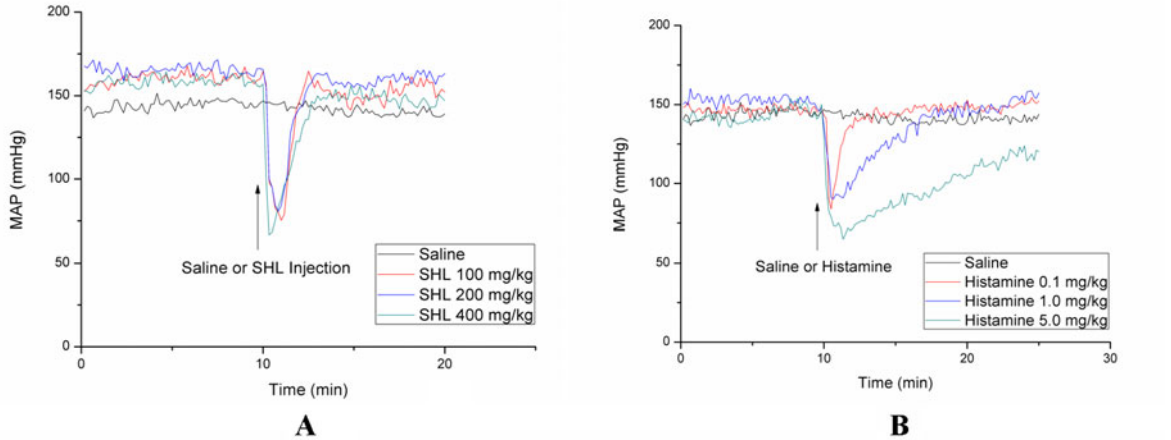

Figure 2. Effect of SHL (A) and histamine (B) on MAP. (**A**) SHL was injected (100, 200 and 400 mg/kg) *via* the jugular vein 10 min after the initiation of MAP monitoring. Results are presented as the average from five rats. (**B**) Histamine was injected (0.1, 1.0, and 5.0 mg/kg) *via* the jugular vein 10 min after the initiation of MAP monitoring. Results are presented as the average from five rats.

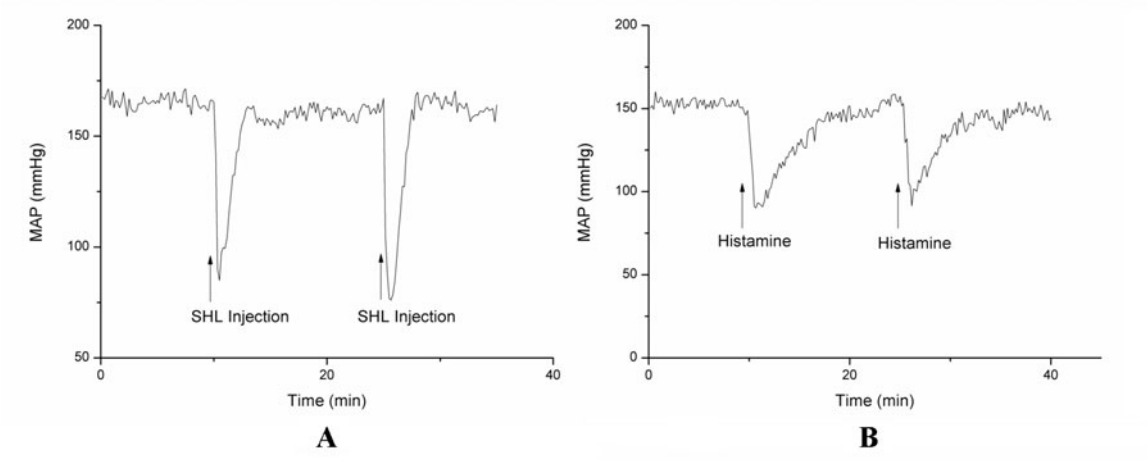

Figure 3. Effect of SHL (A) and histamine (B) on MAP. (**A**) SHL was injected (200 mg/kg) *via* the jugular vein 10 min and 25 min after the initiation of MAP monitoring. Results are presented as the average from five rats. (**B**) Histamine was injected (1.0 mg/kg) *via* the jugular vein 10 min and 25 min after the initiation of MAP monitoring. Results are presented as the average from five rats.

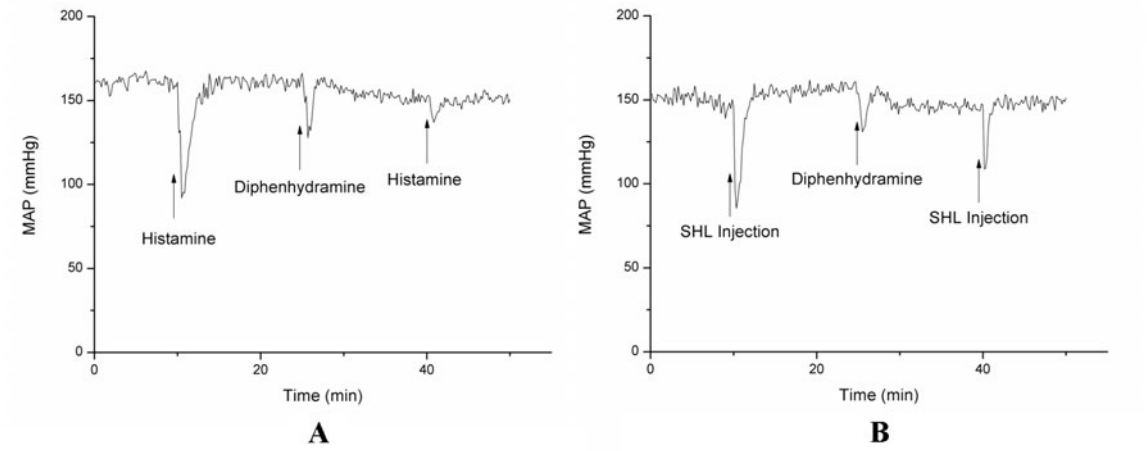

Figure 4. Effects of diphenhydramine and histamine (A) and SHL (B) on MAP. (**A**) Histamine was given in a dose of 200 mg/kg *i.v.* by bolus injection. Fifteen minutes later, diphenhydramine (4.6 mg/kg) was injected as a bolus. Fifteen minutes later, histamine was given again in the same dose as before. Results are presented as the average from five rats. (**B**) SHL was given in a dose of 200 mg/kg *i.v.* by bolus injection. Fifteen minutes later, diphenhydramine (4.6 mg/kg) was injected as a bolus. Fifteen minutes later, SHL was given again in the same dose as before. Results are presented as the average from five rats.

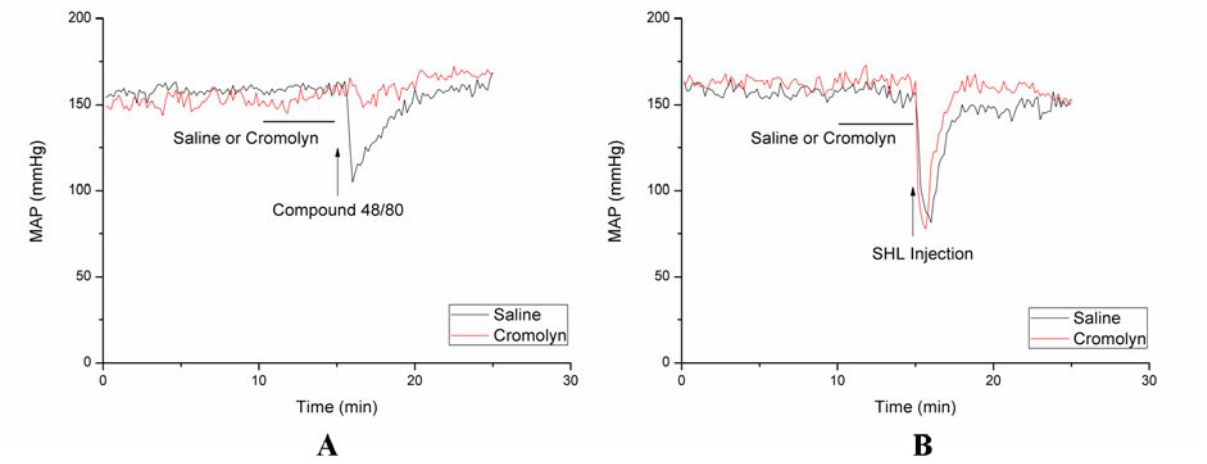

Figure 5. Effect of Cromolyn/saline and Compound 48/80 (A) and SHL (B) on MAP. (**A**) Cromolyn in a dose of 4 mg/kg/min or saline in a dose of 0.1 mL/min (n = 5) was infused for 5 min *via* the jugular vein. Compound 48/80 in a dose of 0.1 mg/kg *i.v.* was given (bolus) immediately after the cromolyn or saline infusion. Results are presented as the average from five rats. (**B**) Cromolyn in a dose of 4 mg/kg/min or saline in a dose of 0.1 mL/min (n = 5) was infused for 5 min *via* the jugular vein. SHL in a dose of 200 mg/kg *i.v.* was given (bolus) immediately after the cromolyn or saline infusion. Results are presented as the average from five rats.

cromolyn, $p < 0.003$). Figure 5B shows the effect of cromolyn on the hypotensive response induced by SHL. When saline was used (0.1 mL/kg/min, 5 min) prior to the injection of SHL (200 mg/kg), MAP decreased rapidly from 156 ± 4.6 mmHg to 81 ± 6.9 mmHg ($p < 0.00005$). Pretreatment with cromolyn did not attenuate the hypotensive response induced by SHL, as evident by 77.6 ± 4.6 mmHg *vs.* baseline 163.4 ± 4.6 mmHg ($p < 0.0002$) (change in MAP from its baseline to 16 min, saline *vs*. cromolyn, $p = 0.16$).

3.2. *Histamine release*

In a separate experiment, blood samples were collected 5 min after the injection (bolus) of saline, compound 48/80, or SHL in order to detection plasma histamine levels. The compound 48/80 and SHL both resulted in an increase in plasma histamine levels (Figure 6).

4. Discussion

As is well-known, the direct release of histamine may cause an anaphylactoid reaction, and physicians consider the plasma histamine level to be the 'golden standard' in gauging such reactions in clinical settings. To determine if an anaphylactoid reaction is imminent, measurement of the plasma histamine level has to be combined with observation of symptoms, *e.g.* skin reaction, tachycardia or bradycardia, hypertension or hypotension, bronchospasms, vomiting, diarrhea, and heart attack or myocardial infarction; such a combination of techniques is commonly used in diagnostic trials and preclinical studies (*13,16*). The current study injected a standard form of exogenous histamine and SHL in order to determine whether SHL causes an anaphylactoid reaction and explore possible mechanisms of that reaction.

Intravenous administration of SHL led to an elevated plasma histamine level and significant hypotension (Figures 2A and 6). When SHL was given to rats intravenously, the MAP decreased rapidly. Increasing doses of SHL from 100 to 400 mg/kg did not increase the extent of hypotension (Figure 2A). Similarly, the injection of histamine led to a MAP reduction immediately after administration. The extent of hypotension was not affected by doses of histamine from 0.1 to 1.0 mg/kg, as was found in a previous study of histamine-mediated hypotension (*13*). Though the response caused by histamine lasts longer than that caused by SHL, the responses are quite similar. Furthermore, the hypotension induced by histamine and SHL was partially inhibited by diphenhydramine, an H_1 receptor antagonist, in a similar manner. Given characteristics identical to the hypotension produced by histamine and evidence of an elevated plasma histamine level after intravenous administration, the hypotension caused by SHL may be histamine-mediated, and the underlying mechanism may be the stimulation of histamine release, which then subsequently induces a hypotensive response. That said, the hypotension induced by SHL was not completely blocked by diphenhydramine (Figure 4), suggesting that other mechanisms that lower the MAP may be at work besides a histamine-related anaphylactoid reaction.

The compound 48/80, a polybasic substance, is known to induce hypotension by stimulating histamine release (*17*), and this hypotension can be attenuated by cromolyn through inhibition of mast cell degranulation (*10*). In the current study, hypotension was attenuated when cromolyn was given prior to administration of the compound 48/80, which did not occur prior to administration of SHL (Figure 5). Presumably, the mechanisms by which SHL and compound 48/80 reduce the MAP differ. Certain substances in the SHI injection may induce a hypotensive response, similar to that induced by histamine, that cannot be blocked by cromolyn. This histamine-like reaction may enhance the hypotensive effects of histamine release (Figure 6). Iizuka T *et al.* found that an extract isolated from the leaves of Forsythia viridissima has a vasorelaxant effect (*18*), and this may play a role in the hypotensive response induced by SHL. The molecule mechanisms responsible are being investigated.

In conclusion, SHL can induce severe hypotension through histamine release in combination with the direct effects of its histamine-like substances on target tissue. An elevated plasma histamine level and hypotension are both observed after intravenous administration, so SHL likely has the potential to cause an anaphylactoid reaction.

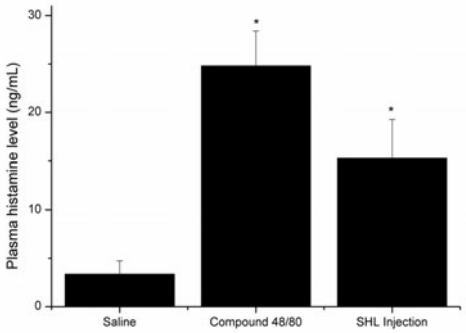

Figure 6. Plasma histamine level. Animals ($n = 5$) were treated with saline, compound 48/80 (0.1 mg/kg), or SHL (400 mg/kg). Blood samples were collected 5 min after the administration. Histamine concentration was measured using an ELISA kit. Results are presented as the average from five rats.* $p < 0.01$, *vs*. saline group.

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