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Hypotensive effect and toxicology of total alkaloids and veratramine from roots and rhizomes of *Veratrum nigrum* L. in spontaneously hypertensive rats

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Total alkaloids (VTA) and veratramine of *Veratrum nigrum* L. were tested for hypotensive effect using spontaneously hypertensive rats (SHR). Acute toxicities were also evaluated. There was a dose-dependent reduction in blood pressure and heart rate after a single ingestion (1.0 to 4.0 mg/kg, intragastric administration) of VTA. A single oral ingestion (0.56 to 2.24 mg/kg) of veratramine, the major component of VTA, dose-dependently decreased blood pressure and heart rate, suggesting that veratramine was involved in the hypotensive effect of VTA in SHR. The hypotensive effects of VTA and veratramine are directly positively correlated with the dosage. Side effects were not obvious.

1. Introduction

The genus *Veratrum* (Family: Liliaceae) has been found to be a rich source of new and bioactive steroidal alkaloids, some of which are well known for their pharmacological potential (Atta-ur-Rahman and Choudhary 1997, 1998). In China, the dried roots and rhizomes of *Veratrum nigrum* L. have been used to treat aphasia arising from apoplexy, wind-type dysentery, jaundice, headache, scabies, chronic malaria, and so forth (Jiangsu College of New Medicine 1986). From *V. nigrum* L. a folk medicine in general use in Jilin Province, China (Jiangsu College of New Medicine 1986), steroidal alkaloids (Tezuka et al. 1998; Li et al. 2007, 1998; Zhao 1987; Liu et al. 1966; Zhao et al. 1991a), stibenoids (Zhao et al. 1998), and peptides (Zhao et al. 1991b, 1998) have been isolated. Pharmacological evaluation of *V. nigrum* L. has revealed hypotensive properties (Li et al. 2000; Liu et al. 1966), a protective effect on ischemia-reperfusion injury in rat liver (Wang et al. 2007), and toxic effects (Lin and Gao 1992).

The hypotensive action of VTA has been documented, but the alkaloid responsible for this activity has not yet been verified, and so VTA and veratramine (the major component of VTA) were used in this study of their hypotensive effects. Acute toxicity tests were also performed to establish the safety of VTA and veratramine.

2. Investigations, results and discussion

2.1. Effects of veratranine and VTA on blood pressure

High blood pressure is a major risk factor for stroke, coronary heart disease and renal vascular disease. The current and usual method for controlling hypertension is long-term drug therapy. Drugs may have side effects which may complicate the patient's medical condition. Traditional physicians and even patients prefer and tend to use older herbal medicines. A considerable number of hypotensive plants and herbs are known through folklore but their introduction into modern therapy awaits pharmacological testing by modern research methods. The pharmacological properties shown here serve to explain the wide use of *V. nigrum* roots and rhizomes by the Chinese population to treat hypertension.

Veratramine is a known veratramine C-nor-D-homosteroidal *Veratrum* alkaloid (Agrawal et al. 1991). It antagonizes the Na^+ channel-gating mechanism of ceveratrum alkaloids by blocking Na^+ channels (Honerjager 1982). Veratramine also shows serotonin (5-HT) agonist activity, acting on presynaptic 5-HT neurons. The administration of vertatramine induces generalized tremors, myoclonus, hindlimb abduction, backward gait, and Straub tail, similar to the 5-HT syndrome in mice (Nagata and Izumi 1991; Izumi et al. 1978). Veratramine has more recently been reported to induce hemolysis of human red blood cells (Badria et al. 1995; El sayed 1998). Veratramine causes bradycardia and periodic rhythm in the sinoatrial node of the guinea pig (Thron and McCann 1998, 1999). However, the hypotensive effect of veratramine is reported here for the first time.

The effects of veratramine on systolic blood pressure and heart rate in controls and veratramine-treated rats are compared in Tables 1 and 2. There was a significant difference between the basal blood pressure of control and veratramine-treated rats. Therefore, veratramine was demonstrated to be the compound responsible for the hypotensive activities of this plant.

Table 1: Effect of veratramine an decreasing SBP during on 8-hour experimental period in spontaneously hypertensive rats (n = 10, X ± SD)

Groups	Doses (mg/kg)	Before administration (Kpa)	After administration (Kpa)							
			0.5 h	1 h	1.5 h	2 h	3 h	4 h	6 h	8 h
Control		22.2 ± 1.01	22.6 ± 1.03	22.4 ± 1.84	22.4 ± 1.20	22.9 ± 2.02	22.5 ± 1.45	23.1 ± 1.17	23.2 ± 2.13	21.9 ± 1.74
			1.64 ± 5.30	0.90 ± 7.77	1.04 ± 5.47	2.84 ± 7.14	1.49 ± 5.63	4.19 ± 5.64	4.53 ± 8.69	−1.37 ± 8.39
Lacidipine	0.36	22.2 ± 1.54	15.2 ± 2.42***	15.8 ± 3.29***	16.8 ± 2.53***	17.8 ± 3.05***	19.8 ± 3.61	20.2 ± 3.12	21.1 ± 2.11	21.6 ± 2.34
			−31.5 ± 9.07***	−29.4 ± 11.6***	−24.5 ± 8.31***	−19.9 ± 10.4***	−11.1 ± 11.7**	−9.38 ± 10.9**	−4.85 ± 6.51*	−2.74 ± 8.73
Veratramine	2.24	22.4 ± 1.07	20.8 ± 2.87	21.8 ± 2.52	20.7 ± 1.28**	21.8 ± 2.39	23.1 ± 2.45	22.8 ± 2.21	23.5 ± 3.13	21.8 ± 1.97
			−6.90 ± 12.6	−2.46 ± 9.20	−7.44 ± 4.50**	−2.36 ± 9.23	3.00 ± 8.58	2.17 ± 9.46	5.09 ± 13.6	−2.01 ± 8.62
Veratramine	1.12	22.1 ± 0.716	21.9 ± 2.82	21.1 ± 1.88	21.0 ± 1.11*	22.1 ± 2.84	21.6 ± 2.49	22.3 ± 2.36	22.6 ± 3.17	22.0 ± 2.44
			−0.961 ± 12.5	−4.88 ± 7.87	−4.89 ± 5.62*	−0.530 ± 11.0	−2.48 ± 10.8	0.740 ± 10.4	1.96 ± 14.4	−0.544 ± 11.2
Veratramine	0.56	22.3 ± 1.35	22.8 ± 2.37	22.3 ± 2.14	22.7 ± 1.57	21.7 ± 2.18	23.1 ± 1.70	22.7 ± 1.48	24.2 ± 1.13**	22.2 ± 1.53
			2.10 ± 9.96	−0.372 ± 6.22	2.02 ± 7.34	−2.74 ± 8.20	3.45 ± 6.63	1.72 ± 5.70	8.79 ± 8.28	−1.02 ± 6.80

*p < 0.05; **p < 0.01; ***p < 0.001

Table 2: Effect of veratramine on decreasing heart rate during an 8-hour experimental period in spontaneously hypersentive rats (n = 10, X ± SD)

Groups	Doses (mg/kg)	Before administration (b/m)	After administration (b/m)							
			0.5 h	1 h	1.5 h	2 h	3 h	4 h	6 h	8 h
Control		462 ± 8.04	454 ± 21.4	454 ± 16.0	449 ± 22.6	452 ± 17.2	453 ± 19.6	454 ± 25.2	450 ± 25.6	450 ± 20.0
			−1.56 ± 4.34	−1.76 ± 3.56	−2.74 ± 5.39	−2.10 ± 4.17	−1.95 ± 4.93	−1.67 ± 5.36	−2.63 ± 5.86	−2.85 ± 4.62
Lacidipine	0.36	460 ± 16.0	470 ± 19.1	461 ± 46.1	455 ± 36.5	451 ± 31.8	452 ± 30.8	432 ± 32.2*	450 ± 44.4	450 ± 31.8
			2.35 ± 4.94	0.259 ± 9.72	−1.13 ± 6.00	−1.98 ± 6.85	−1.64 ± 7.26	−6.05 ± 6.33	−2.13 ± 8.43	−0.765 ± 5.92
Veratramine	2.24	453 ± 23.3	448 ± 42.0	452 ± 34.6	437 ± 30.0	442 ± 30.3	411 ± 40.1*	419 ± 26.5**	423 ± 31.9*	422 ± 27.6*
			−0.624 ± 12.2	−0.09 ± 7.92	−3.53 ± 4.53	−2.15 ± 6.89	−9.26 ± 6.23**	−7.15 ± 7.23	−6.23 ± 8.83	−6.59 ± 8.17
Veratramine	1.12	458 ± 11.5	442 ± 20.8*	443 ± 26.7	437 ± 26.0*	424 ± 41.9*	413 ± 48.1*	424 ± 29.6**	422 ± 36.7**	438 ± 24.5*
			−3.45 ± 4.51	−3.17 ± 5.40	−4.46 ± 5.26	−7.41 ± 8.29	−9.75 ± 9.80*	−7.15 ± 7.19	−7.65 ± 8.16	−5.16 ± 5.01
Veratramine	0.56	460 ± 14.3	446 ± 43.4	440 ± 18.4*	441 ± 27.3	443 ± 32.6	442 ± 29.9	424 ± 36.8*	435 ± 28.7*	425 ± 35.0**
			−2.92 ± 9.24	−4.28 ± 4.73	−3.95 ± 5.05	−3.59 ± 6.04	−3.94 ± 5.05	−7.68 ± 7.10*	−5.31 ± 6.15	−7.17 ± 8.02

*p < 0.05; **p < 0.01; ***p < 0.001

The present study demonstrates that VTA and veratramine are effective in reducing blood pressure and heart rates in SHR rats. This effect is shown to be dose-related and rapid in onset.

The synergistic effect of VTA can be attributed to the presence of veratramine and other steroidal alkaloids. The main class of compounds isolated from *V. nigrum* L. is steroidal alkaloids, which can be divided into five types on the basis of the carbon framework: veratramine, jervine, cevanine, solanidine and verazine. These five types of steroidal alkaloids may be different not only in terms of potency, but also at least partially in terms of their mechanism of action, and this may explain their synergistic effect observed when they are tested in combination in VTA. Further studies with other pure steroidal alkaloids and other experimental animal models (anesthetized normotensive and renal hypertensive rats) will be required in order to elucidate the mode of action to fully explain the antihypertensive activity shown by the complex of VTA obtained from *V. nigrum* L.

The above findings may partly justify the rationale for the use of *V. nigrum* L. in folk medicine in China, for the treatment of suspected hypertensive patients. Although its mechanism is still unknown, VTA and veratramine seem to be effective and safe antihypertensive phytomedicines.

2.2. Acute toxicity

The LD_{50} of VTA was calculated to be 23.5 mg/kg, and the LD_{50} of veratramine was calculated to be 15.9 mg/kg.

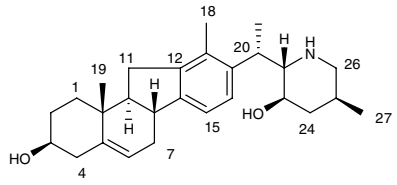
2.3. Effect of VTA on systolic blood pressure and heart rate in spontaneously hypertensive rats

At the initiation of the study, the systolic blood pressure (SBP) in 50 rats were measured by the tail-cuff method every day for a week using a blood pressure-monitor. The mean initial SBP was calculated as the base value. Mean blood pressure and heart rate ± S.E.M. of control and treated rats after oral administration of different doses of VTA at various time intervals are summarized in Tables 3 and 4. After 0.5 h treatment, the effect of oral administration of VTA on blood pressure was examined. Systolic blood pressure in ten rats in each group was measured by the tail-cuff method, using a blood pressure-monitor. The maximum and dose-related effect can be observed 2 h after treatment with VTA. The results obtained in the present study showed that administration of VTA to SHR rats, at dose levels of 2.0 and 4.0 mg/kg, produced a significant decrease in blood pressure between 0.5 and 2 h after oral administration. Treatment with lacidipine 0.36 mg/kg orally, caused a highly significant reduction in blood pressure levels up to 3 h. The heart rate was decreased by VTA as shown Table 2, while lacidipine had the side effect of accelerating heart rate.

2.4. Effect of veratramine on systolic blood pressure and heart rates in spontaneously hypertensive rats

SBP and heart rate after oral administration of veratramine in SHR are shown in Tables 1 and 2. The initial SBP values were 22.2 ± 1.01, 22.2 ± 1.54 Kpa in the negative and positive control groups, and 22.3 ± 1.35, 22.1 ± 0.716, and 22.4 ± 1.07 Kpa in the 0.56, 1.12, and 2.24 mg/kg veratramine groups, respectively. There were no significant changes in SBP in the negative control distilled water group throughout the experimental period. Veratramine significantly decreased blood pressure in a dose-dependent manner, the changes in SBP 1.5 h after administration being 4.89 and 7.44% in the 1.12 and 2.24 mg/kg veratramine groups, respectively. The reduction in blood pressure persisted for 2 h after administration. The initial heart rates were 462 ± 8.04 (beats/min) in the negative control distilled water group; the heart rate was slowed down by veratramine after oral administration as shown in Table 2, while the heart rate was accelerated by lacidipine after oral administration.

2.5. Identification of veratramine



Veratramine was identified by comparing spectral data with those reported (Tezuka et al. 1998; Atta-ur-Rahman et al. 1991). Veratramine formel colorless crystal line needles; melting point: 214.2–216.5 °C; $[\alpha]_D^{25}$D-55.9 ($CHCl_3$, c = 0.5); IR (KBr) cm^{-1}, 3311, 2900, 2927, 2844, 1670, 1458, 1062, 962, 813; UV: 220, 268, 270 nm; EI-MS(m/z): 410$[M + H]^+$; ^{1}H-NMR ($CDCl_3$, 500 MHz): 0.83 (3 H, d, J = 7.0 Hz, 27-H), 1.15 (3 H, s, 19-H), 1.40 (3 H, d, J = 7.5 Hz, 21-H), 2.11 (1 H, brs, 20-H), 2.32 (3 H, s, 18-H), 2.50(1 H, dd, J = 9.0, 4.0 Hz, 22-H), 3.27 (1 H, m, 23-H), 3.52 (1 H, m, 3-H), 5.49 (1 H, brd, J = 4.0 Hz, 6-H), 6.97 (1 H, d, J = 7.5 Hz, 15-H), 7.22 (1 H,d, J = 7.5 Hz, 16-H); ^{13}C-NMR ($CDCl_3$): 37.3 (C-1), 29.5 (C-2), 70.1 (C-3), 41.1 (C-4), 141.8 (C-5), 120.5 (C-6), 40.3 (C-7), 43.5 (C-8), 56.1 (C-9), 36.0 (C-10), 29.6 (C-11), 139.5 (C-12), 131.9 (C-13), 142.6 (C-14), 118.7 (C-15), 124.7 (C-16), 142.2 (C-17), 15.0 (C-18), 18.1 (C-19), 31.4 (C-20), 18.4 (C-21), 66.3 (C-22), 69.6 (C-23), 34.6 (C-24), 30.4 (C-25), 53.2 (C-26), 19.7 (C-27).

3. Experimental

3.1. General experimental procedures

The melting point was measured on an X4 micro-melting point apparatus and was uncorrected. IR spectra were recorded in KBr disks on a Nicolet Impact 410 spectrophotometer. NMR spectra were obtained in $CDCl_3$ containing TMS as an internal standard on a Bruker Am-500 NMR spectrometer. EI-MS spectra were performed on an EI-TOF mass spectrometer (Agilent, U.S.A.).

3.2. Plant material

The roots and rhizomes of *V. nigrum* L. were collected in Antu Prefecture, Jilin Province, China in June 2002, and a voucher specimen (VN2002003) was taxonomically certified by Professor Minglu Deng, College of Pharmacy, Changchun University of Chinese Medicine, P. R. China.

3.3. Animals

Kunming mice of both sexes, weight 19–21g, aged 8–10 weeks were used. They were obtained from the Changchun High-Tec Medical Animal Center. Male SHR (SHR/NCrj) rats purchased from Charles River China, Inc. (Beijing, China) were used. All animals were maintained at a temperature of 25 ± 1 °C, 55 ± 10% humidity, and 12 h on/off light cycle (7:00 AM–7:00 PM). The animals had unlimited access to water throughout the period of the study. They were allowed to acclimatise under climate-controlled conditions for a week before use. Animal experiments were conducted in accordance with current ethical regulations for animal care and use at Changchun University of Chinese Medicine.

Table 3: Effect of VTA on decreasing SBP during an 8-hour experimental period in spontanously hypertensive rats (n = 10, X ± SD)

Groups	Doses (mg/kg)	Before administration (Kpa)	After administration (Kpa)							
			0.5 h	1 h	1.5 h	2 h	3 h	4 h	6 h	8 h
Control		22.3 ± 0.85	22.7 ± 3.18	21.0 ± 3.40	22.0 ± 3.77	21.6 ± 3.15	21.6 ± 2.97	21.3 ± 3.68	21.5 ± 2.08	21.7 ± 2.49
			1.40 ± 11.9	−6.07 ± 13.9	−1.84 ± 14.8	−4.94 ± 11.6	−3.09 ± 13.4	−4.70 ± 15.3	−3.73 ± 8.90	−2.62 ± 11.8
Lacidipine	0.36	22.5 ± 1.10	10.8 ± 2.73***	12.1 ± 2.53***	14.5 ± 3.38***	15.9 ± 2.41***	17.0 ± 4.00***	19.0 ± 3.35**	19.8 ± 3.19*	20.7 ± 2.94
			−52.2 ± 11.0***	−46.2 ± 10.8***	−35.8 ± 13.7***	−29.2 ± 11.1***	−24.8 ± 16.1**	−15.6 ± 12.9	−11.9 ± 13.0	−7.82 ± 13.2
VTA	4	22.5 ± 1.09	16.3 ± 4.76***	13.7 ± 3.02***	16.2 ± 3.93***	16.9 ± 3.39***	19.8 ± 3.23*	20.2 ± 2.23**	20.8 ± 1.70*	21.4 ± 1.88
			−28.3 ± 17.8***	−39.1 ± 14.7***	−27.6 ± 18.8**	−25.1 ± 14.7**	−12.5 ± 12.3	−10.3 ± 7.69	−7.69 ± 5.85	−5.13 ± 7.35
VTA	2	22.2 ± 0.72	17.1 ± 2.51***	17.1 ± 1.71***	18.0 ± 1.82***	18.3 ± 2.34***	20.4 ± 2.98	20.3 ± 1.66**	22.0 ± 1.98	22.2 ± 2.14
			−22.7 ± 11.0***	−22.7 ± 7.18**	−18.8 ± 8.28**	−17.6 ± 10.5*	−7.92 ± 13.5	−8.51 ± 8.85	−0.92 ± 8.85	0.21 ± 8.58
VTA	1	22.4 ± 1.09	17.4 ± 3.52***	18.9 ± 3.81*	20.1 ± 3.04*	19.8 ± 2.72*	21.4 ± 3.70	21.3 ± 2.22	21.0 ± 2.81	21.7 ± 1.71
			−22.6 ± 14.1***	−15.5 ± 17.1	−10.7 ± 9.45	−12.0 ± 9.94	−4.99 ± 13.7	−4.94 ± 7.32	−5.89 ± 12.8	−2.93 ± 8.53

*p < 0.01; **p < 0.01; ***p < 0.001

Table 4: Effect of VTA on decreasing heart rate during an 8-hour experimental period in spontaneously hypertensive rats (n = 10, X ± SD)

Groups	Doses (mg/kg)	Before administration (b/m)	After administration (beat/minute)							
			0.5 h	1 h	1.5 h	2 h	3 h	4 h	6 h	8 h
Control		456 ± 10.6	465 ± 22.4	461 ± 35.3	438 ± 31.4	432 ± 12.0	428 ± 30.5	432 ± 42.3	443 ± 26.6	427 ± 13.7
			2.05 ± 5.07	1.10 ± 7.44	−3.89 ± 6.65	−5.20 ± 3.10	−6.14 ± 6.90	−5.33 ± 8.66	−2.84 ± 6.27	−6.30 ± 2.56
Lacidipine	0.36	463 ± 17.0	491 ± 27.2*	492 ± 29.8*	455 ± 50.8	465 ± 31.4	451 ± 32.0	445 ± 22.7	441 ± 31.3	443 ± 22.5*
			6.32 ± 7.29	6.38 ± 5.95	−1.48 ± 11.9	0.42 ± 5.48*	−2.44 ± 7.16	−3.71 ± 5.69	−4.48 ± 7.84	−4.20 ± 6.11
VTA	4	452 ± 20.6	394 ± 32.5***	368 ± 72.8**	395 ± 37.4***	407 ± 38.6**	443 ± 21.8	430 ± 11.8**	432 ± 13.4*	437 ± 23.9
			−12.9 ± 6.07***	−19.0 ± 14.2***	−12.6 ± 6.98*	−10.1 ± 6.49*	−1.87 ± 7.89	−4.68 ± 6.02	−4.26 ± 6.16	−3.19 ± 8.71
VTA	2	458 ± 10.2	418 ± 26.4***	434 ± 35.9	423 ± 26.0***	421 ± 38.9**	421 ± 39.5**	436 ± 37.8	436 ± 19.5**	439 ± 29.7
			−8.75 ± 6.31***	−5.27 ± 8.42	−7.55 ± 6.00	−8.14 ± 7.83	−8.22 ± 7.70	−4.68 ± 8.19	−4.62 ± 5.31	−3.98 ± 7.64
VTA	1	457 ± 15.6	416 ± 52.9*	432 ± 42.6	432 ± 18.6**	426 ± 37.8*	427 ± 28.5**	435 ± 35.7	448 ± 28.9	442 ± 15.2*
			−8.86 ± 12.1*	−5.64 ± 7.75	−5.55 ± 3.56	−6.79 ± 7.39	−6.67 ± 4.65	−4.95 ± 5.45	−1.89 ± 6.73	−3.30 ± 3.96

*p < 0.01; **p < 0.01; *** p < 0.001

3.4. Reference drug

Lacidipine tablets (Harbin Pharm. Group Sanjing Pharmaceutical Shareholding Co. Ltd., China) were used as the reference drug (positive control). It was dissolved in distilled water prior to administration.

3.5. Extraction and isolation

The finely powdered roots and rhizomes of *V. nigrum* L. (2 kg) were soaked for maceration in 0.4% NaOH for half an hour and then were extracted thrice with $CHCl_3$ for 2 days each time at room temperature, yielding 105 g of a dark brown tarry mass after evaporation of the solvent. The mass was acidified (pH = 4) with 5% tartaric acid, followed by filtration, and the acidic aqueous filtrate was then basified with NH_4OH to pH = 9, to allow alkaloids to precipitate out from the solution. Finally, the alkaloids (VTA) were obtained after filtration of the solution and drying the precipitate at 55 °C. The solid residue (51 g) was maintained at −4 °C overnight, and the filtrate was extracted with $CHCl_3$/MeOH (9 : 1) to give a solid residue of VTA (12 g), which when recrystallized form MeOH, yielded veratramine (350 mg), one of the major components of the total alkaloids. The total alkaloid content was 90%, determined by a colorimetrical method, veratramine being used as standard.

3.6. Acute toxicities of VTA and veratramine in mice

Kunming male and female mice (19–21g) were housed in cages at 22 °C. Prior to the experiments, they were starved overnight with free access to water. Seven groups of 10 animals each containing an equal number of males and females were formed. Intragastric doses of 34.1, 30.0, 26.4, 23.2, 20.4, 18.0, and 16.2 mg/kg of VTA were prepared and suspended in vehicle (Tween-80, 0.2% in distilled water), and administered intragastrically to the animals in the test groups. In each case the volume administered was 20 mL/kg. After administration of the product, animals were closely observed for the first 9 min, and at intervals thereafter, for 7 days, for toxicity symptoms or death. The weight of each animal was measured to obtain the body weight loss or gain. After a 7-day experimental period, the mice were sacrificed and the vital organs were macroscopically observed. Mice were observed daily over a period of 7 days for mortality, toxic effects and/or changes in behavioral pattern. At the end of the experiments the animals were sacrificed in a CO_2 chamber. Using the same procedure, intragastric doses of 25.5, 21.7, 18.4, 15.7, 13.3, 11.3, 9.6 mg/kg of veratramine were prepared and administrated.

3.7. Blood pressure and heart rate of spontaneously hypertensive rats

In an attempt to validate the hypotensive properties attributed to *V. nigrum* L., a biological evaluation of total alkaloids and veratramine of *V. nigrum* L. was carried out using on experimental model. Blood pressure and heart rate were measured using the tail-cuff method in conscious SHR rats. After warming in a warmer at 37 °C for 15 min, the rats were placed in a holder, and the blood pressure and heart rate of the tail artery were measured using an automatic blood pressure monitoring system (RBP-1; Institute of Clinical Medical Research, China-Japan Friendship Hospital).

3.8. Statistical analysis

Statistical evaluation of the results was done using Student's t-test to determine the significance of difference in the mean values; the values were expressed as means ± S.E.M. (standard error of means). Significant differences were indicated by P values.

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