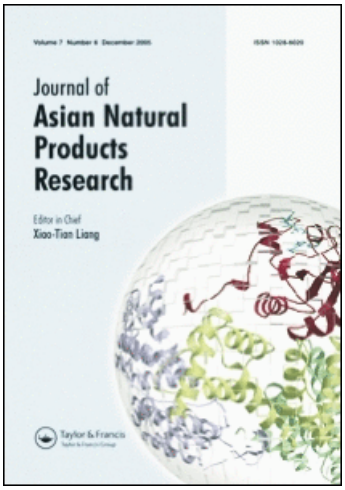

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A new semisynthetic ocotillol-type saponin and resuscitation of haemorrhagic shock

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A new ocotillol-type saponin (PGQ) has been semisynthesised from 20(S)-ginsenoside Rg3 for the first time, in with a yield of 73%, by oxidation and cyclisation of the side-chain. Its structure was characterised by IR, HR-MS, ^{1}H NMR, ^{13}C NMR, ^{1}H-^{1}H COSY, DEPT, HMBC and HMQC spectroscopy as 3-*O*-[β-D-glucopyranosyl-(1-2)-β-D-glucopyranosyl]-dammar- 20S,24S-epoxy-3β,12β,25-triol (**1**). The saponin produced a beneficial effect on resuscitation of haemorrhagic shock as follows: first, it could significantly rise increased mean arterial pressure; second, it could increased blood oxygen content; third, it could markedly decreased serum lipoperoxidase and increased superoxide dismutase (SOD) activities. Thus, the saponin had the effect of an anti-haemorrhagic shock.

Keywords: Haemorrhagic shock; Ocotillol-type saponin; Resuscitation; Semisynthesis; 3-*O*-[β-D-glucopyranosyl-(1-2)-β-D-glucopyranosyl]-dammar-20S,24S-epoxy-3β,12β,25-triol; Haemorrhagic shock

1. Introduction

Both *Panax ginseng* and *Panax quinquefolium,* belonging to the Araliaceae, are well know traditional medicinal herbs. They are used as tonics and for the treatment for many diseases. *Panax ginseng* contains numbers of saponins, called ginsenosides, including an oleanolic acid-type saponin in addition to the major protopanaxadiol and protopanaxatriol-type saponins [1]. However, *Panax quinquefolium* contains an ocotillol-type (20,24-epoxysides) saponin with high activity [2], as well as oleanolic acid-type, protopanaxadiol and protopanaxatriol-type saponins. Therefore, to obtain the ocotillol-type saponin by modifying protopanaxadiol and protopanaxatriol-type saponins is an interesting study. In this paper, we report the synthesis of a new ocotillol-type saponin by the oxidation and cyclisation of the side-chain of 20(S)-ginsenoside Rg3. In the pharmacological experiments, we found that this new compound had the effect of anti-haemorrhagic shock in a canine model of haemorrhagic shock. To achieve this objective, the changes in mean arterial pressure (MAP), blood oxygen content, and lipoperoxidase (LPO) and superoxide dismutase (SOD) activities in anaesthetised dogs were determined before and during shock. The results showed that the compound could significantly increase MAP and increase blood oxygen content, and it could markedly decrease serum LPO and increase SOD activities compared with the control group.

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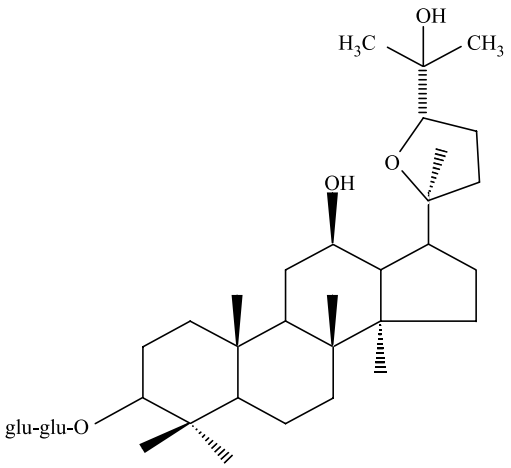
2. Results and discussion



Scheme 1. 20(S)-ginsenoside Rg3.

The isolation of the individual compounds in a pure state from crude extracts, especially minor components, is often tedious. Due to the biological activity and the low content of some dammarane glycosides from ginseng, the preparation is of interest [3]. In particular, this includes ginsenoside Rg3, a minor component isolated from ginseng, which has an anti-cancer effect [4]. We obtained 20(S)-ginsenoside Rg3 (**2**) by mild acidic hydrolysis of ginseng panaxadiol group saponins (including ginsenoside Rb, Rc, Rd, etc.) with a high yield (10%), and we have obtained a patent on this method in China. Therefore, we focused on transforming 20(S)-ginsenoside Rg3 to a new ocotillol-type saponin during the course of cyclisation of the side-chain with the purpose of improving the solubility and exploiting pharmacological activities.

It is known that 12β,20-dihydroxydammarane-type triterpenes are easily effected by acid-catalysed epimerisation to yield a mixture of their 20S- and 20R-epimers [5]. Furthermore, it has been reported that acid treatment of a cycloartane compound having a 20,24,25-trihydroxylated side-chain yielded its 20,24-epoxy-compound [6]. *m*-Chloroperbenzoic acid is a useful oxidising reagent for epoxidation [7]. Thus, the new ocotillol saponin was semisynthesised in an independent procedure from 20(S)-ginsenoside Rg3 by oxidation with *m*-chloroperbenzoic acid in $CHCl_3$.

The new ocotillol-type saponin was obtained as white amorphous powder, and quasimolecular ion peak at *m/z* 824.0212 (M + Na) in the HR-MS showed its molecular formula to be $C_{42}H_{72}O_{14}$. It showed a resemblance with ginsenoside Rg3 in their ^{13}C NMR spectra (125.8 MHz, C_5D_5N). Carbon signals of the new ocotillol saponin were similar to those of 20(S)-ginsenoside Rg3, excluding the signals attributable to the cyclised side-chain. The difference between the two saponins was observed in the side-chain: there were no olefin carbon signals (δ126,δ130 of 20(S)-ginsenoside Rg3) in the new saponin and the side-chain of 20(S)-ginsenoside Rg3 was changed into an epoxy structure. The carbon signals of the cyclised chain were compared with 20S,24S-epoxy-dammarane-3β,6α,12β,25-tetraol and 20S,24R-epoxy-dammarane-3β,6α,12β,25-tetraol [8]. The results showed that the configuration of C-24 of this new ocotillol-type saponin was confirmed as *S*-form.

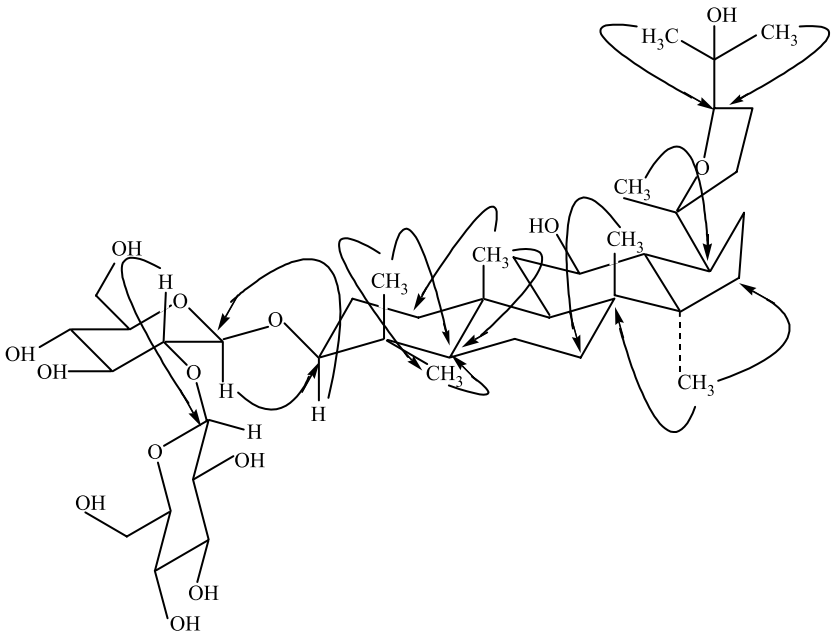


Figure 1. Key HMBC correlations for **1.**

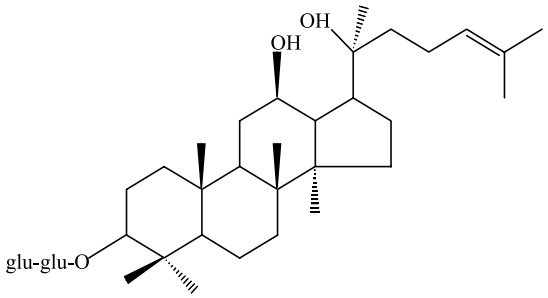
According to the literature, the difference of 20(S)- and 20(R)- in ocotillol-type saponin may be observed from the carbon signal of C-21 (S:δ28 ± 1; R:δ20 ± 1) [5]; in the new compound's spectra, the results showed that the configuration of C-20 of this new saponin was confirmed as *S*-form. The carbon signals according to the reports and the ^{13}C NMR, ^{1}H NMR, HMBC and HMQC spectra are summarised in table 1.

In the HMBC spectrum of this new compound, one signal of H-17 at δ2.30 correlated with the signal of C-17 at δ49.4. In the HMQC spectrum, one proton signal at δ4.16 (dd, J = 7.0, 7.0 Hz) correlated with the carbon signal of C-24 at δ88.3, so the proton signal was attribute to H-24. In the ^{13}C NMR spectrum, the signals of C-22 at δ32.1 and C-23 at δ26.9 were distinguishable, thus, according to the HMQC spectrum, the signal of H-22 at δ1.97, 1.70 and the signal of H-23 at δ2.16, 1.82 were confirmed.

Because the methyl signals of C-30, C-29, C-28, C-27, C-26, C-21, C-19 and C-18 overlapped, confirmation of the proton signals would be difficult by using HMQC spectra. We therefore confirmed their proton signals by using HMBC spectra (figure 1).

By comprehensive analyses of all two-dimensional NMR spectra, the carbon and proton signals of the new ocotillol-type saponin were unequivocally assigned as shown in table 1. The structure of the new compound was characterised as 3-*O*-[β-D-glucopyranosyl-(1-2)-β-D-glucopyranosyl]-dammara-20S,24S-epoxy-3β,12β,25-triol (**1**).

Haemorrhage is one of the major causes of death following trauma, acutely due to blood loss itself, or later in the course of a subsequent metabolic and immunological depression, favouring development of sepsis and/or multiple organ failure. It is well known that haemorrhagic shock and resuscitation represent a typical global ischaemia/reperfusion injury [9]. The primary goal in resuscitation from haemorrhagic shock consists in rapid restoration of the circulating blood volume; lipid peroxidation is a free radical-related process that occurs in biological systems; SOD has been detected in a large number of tissues and organisms, and it is thought to protect



Scheme 2. PGQ.

Table 1. NMR data for **1**.

	δC		Correlated proton		
No.	*Rg3 (**2**)*	***1***	*One-bond*	*Long-range*	*1H-1H COSY*
	39.3	39.2	1.52(1e), 0.79(1a)	19	1e, 2a
2	26.9	26.9	2.18(2e), 1.82(2a)		1a, 3, 2e
3	89.1	88.9	3.27(dd, J = 4.5, 4.5 Hz)	3-glc-1′	2a, 2e
4	39.9	39.6		28,29	
5	56.6	56.4	0.66(d, J = 10.0 Hz)	28,29,19	6a
6	18.6	18.4	1.51(6e), 1.39(6a)		5,7a
7	35.4	35.1	1.37(7e), 1.23(7a)	18	6e, 6a
8	40.2	39.9		18,30	
9	50.6	50.4	1.42	18,19	11e
10	37.1	36.9		19	
11	32.2	32.1	2.03(11e), 1.39(11a)		9,13,12
12	71.2	70.7	3.75 (m)		11a, 13
13	48.8	49.2	1.86(m)	30	11,12,17
14	51.9	52.2		18,30	
15	31.5	32.6	1.51(15e), 1.09(15a)	30	16
16	27.0	28.6	1.94(16e), 1.49(16a)	17	17,15a
17	55.0	49.4	2.30(m)	21	13,16
18	16.0	15.6	1.00(s)	7	
19	16.5	16.5	0.83(s)		
20	73.1	87.0		21	
21	28.3	28.9	1.30(s)		
22	36.1	32.1	1.97(22e), 1.70(22a)	21	23a, 23e
23	23.2	26.7	2.16(23e), 1.84(23a)	27	22a, 22e
24	126.5	88.3	4.16(dd, J = 7.0, 7.0 Hz)	26,27	23a, 23e
25	130.9	69.9		26,27	
26	25.9	25.7	1.30(s)		
27	17.2	28.6	1.45(s)		
28	28.3	28.0	1.28(s)	29,3	
29	16.8	15.6	1.11(s)	28,5	
30	17.8	18.0	0.91(s)		
3-glc-1′	105.3	105.0	4.93(d, J = 8.0 Hz)	3	3-glc-2′
2′	83.7	83.4	4.26	2″-glc-1″	3-glc-1′
3′	78.1	77.9	4.26		
4′	71.8	71.5	4.17		
5′	78.4	78.1	3.93		
6′	63.1	62.8	4.56,4.39		
2″-glc-1″	106.2	106.0	5.37(d, J = 7.5 Hz)	3-glc-2′	2″-glc-2″
2″	77.3	77.0	4.16		2″-glc-1″
3″	78.5	78.3	4.32		
4″	71.9	71.6	4.35		
5″	78.2	78.0	3.94		
6″	62.9	62.7	4.50,4.49		

the cell from damage by superoxide radicals [10]. The aim of this experimental study was to analyse changes in MAP, artery blood oxygen content, and LPO and SOD activities during haemorrhagic shock and resuscitation with the new ocotillol-type saponin in dogs.

Thirty randomised adult dogs were shocked by removing 37 ± 9 ml blood/kg while maintaining MAP < 5.33 kPa. The dogs were divided into five groups ($n = 6$ in each) randomly (group 1: i.v. 0.9% sodium chloride; group 2: i.v. dopamine [5 mg·kg^{-1}]; group 3: i.v. new saponin solution [4 mg·kg^{-1}]; group 4: i.v. new saponin solution [2 mg·kg^{-1}]; group 5: i.v. new saponin solution [1 mg·kg^{-1}]). The new saponin yielded a similar effect to dopamine. Haemorrhagic shock produced a low MAP. The compound produced a significantly greater rise in MAP than in the control sodium chloride group ($p < 0.05$). Haemorrhagic shock produced a moderate increase in LPO activity and a moderate decrease in SOD activity. However, the new saponin decreased LPO activity compared with the control group ($p < 0.05$) 120 min after administration; the LPO activity in the dopamine group was significantly lower ($p < 0.05$) 120 min after administration. SOD activity in the group with the highest saponin intake was significantly higher than in the control group ($p < 0.05$, $p < 0.01$) 120 min after administration; SOD activity in the dopamine group was significantly higher ($p < 0.01$) 120 min after administration. In summary, the present study shows that the saponin had the effect of anti-haemorrhagic shock in a canine model of haemorrhagic shock.

3. Experimental

3.1 General experimental procedures

The melting points were determined on WRS-1B Digital Melting Point Apparatus (Shanghai, China) and are uncorrected. IR data were taken on a AVATAR 330 FT-1R Thermo Nicolet (Maddison, Wisconsin, USA). HR-MS data were taken on a Qstar (Foster City, California, USA). spectrometer, NMR spectra were taken in pyridine-*d5* on a Bruker Avance-500 spectrometer, with TMS as internal standard. All solvent systems for chromatography were homogeneous. For HPLC, an ODS (250 × 10 mm) column was used (solvent 90% MeOH, flow rate 0.5 ml·min^{-1}; Differential Refractometer R401 as detector). For column chromatography, silica gel H (200–300 mesh; Qingdao Marine Chemical Inc., Qingdao City, China) was used. Other equipment included: RM-6000 type multilead physiological recorder (Photoelectric Inc., Japan); 722 type spectrophotometer (Third Apparatus Inc., Shanghai, China); Corning 158 type blood gas analysis (ConAgra Inc., USA).

3.2 Epoxide preparation of glycosides and isolation

To a solution of 0.5 g 20(S)-ginsenoside Rg3 in 10 ml $CHCl_3$, 0.3 g *m*-chloroperbenzoic acid was added at 60°C. After 20 min, the solution was washed with a saturated solution of Na_2CO_3 and dried over Na_2SO_4 [11]; then the reaction mixture was concentrated, and the residue was chromatographed on a column of silica gel with $CHCl_3$-EtOAC-MeOH-H_2O (15:40:22:10) (deposited below 10°C, lower layer) to give three fractions. Fraction 2 was purified by HPLC with the removal solvent (MeOH-H_2O, 9:1). The new ocotillol-type saponin was obtained (0.36 g) by HPLC on ODS with 90% MeOH.

Table 2. The effect on MAP (kPa) of PGQ.

	Sodium chloride group	*Dopamine group* ($5\,mg{\cdot}kg^{-1}$)	*Low-dose drug group* ($1\,mg{\cdot}kg^{-1}$)	*Middle-dose drug group* ($2\,mg{\cdot}kg^{-1}$)	*High-dose drug group* ($4\,mg{\cdot}kg^{-1}$)
Baseline	18.5 ± 2.9	18.9 ± 2.5	18.2 ± 1.4	18.4 ± 2.7	18.5 ± 2.0
Shock	5.4 ± 0.3	5.2 ± 0.2	5.4 ± 0.1	4.9 ± 0.5	5.1 ± 0.2
5 min	6.3 ± 0.9	12.3** ± 3.4	7.3 ± 1.0	7.2 ± 0.9	7.6* ± 0.3
10 min	6.7 ± 1.2	10.3** ± 1.6	7.9 ± 1.3	8.2 ± 1.4	8.3* ± 0.2
20 min	7.2 ± 0.6	9.1* ± 1.3	8.3* ± 1.0	8.5* ± 1.1	9.1*** ± 0.4
30 min	7.1 ± 0.8	9.2 ± 2.3	8.1* ± 0.6	8.7* ± 1.1	9.7*** ± 1.0
45 min	7.3 ± 1.1	7.4 ± 1.2	7.9 ± 1.4	8.15 ± 1.7	9.6* ± 1.4
60 min	7.6 ± 1.1	6.8 ± 1.5	7.3 ± 1.3	7.7 ± 2.1	9.6* ± 2.1
90 min	6.1 ± 1.1	5.9 ± 1.0	7.0 ± 1.4	7.2 ± 2.0	9.7** ± 2.3
120 min	5.5 ± 1.4	5.2 ± 0.5	7.2 ± 2.1	6.8 ± 1.9	9.7** ± 2.6

*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

Table 3. The effect on the arterial blood oxygen content of PGQ.

	Sodium chloride group	*Dopamine group* ($5\,mg{\cdot}kg^{-1}$)	*Low-dose drug group* ($1\,mg{\cdot}kg^{-1}$)	*Middle-dose drug group* ($2\,mg{\cdot}kg^{-1}$)	*High-dose drug group* ($4\,mg{\cdot}kg^{-1}$)
Baseline	18.1 ± 1.7	18.5 ± 0.6	18.5 ± 1.2	18.8 ± 0.9	19.1 ± 1.0
Shock	19.8 ± 0.2	19.5 ± 0.4	19.3 ± 0.5	19.5 ± 0.4	19.7 ± 0.2
60 min	19.9 ± 0.1	20.1* ± 0.1	19.8 ± 0.1	19.9 ± 0.1	19.6 ± 0.6
120 min	19.4 ± 0.5	18.6 ± 2.5	19.2 ± 0.6	20.0* ± 0.1	19.9 ± 0.3

*$p < 0.05$.

Table 4. The effect on the LPO activity of PGQ.

	Sodium chloride group	*Dopamine group* ($5\,mg{\cdot}kg^{-1}$)	*Low-dose drug group* ($1\,mg{\cdot}kg^{-1}$)	*Middle-dose drug group* ($2\,mg{\cdot}kg^{-1}$)	*High-dose drug group* ($4\,mg{\cdot}kg^{-1}$)
Baseline	1.2 ± 0.4	1.4 ± 0.8	1.3 ± 0.3	1.0 ± 0.5	1.5 ± 0.4
120 min	52.9 ± 8.1	40.2* ± 6.0	43.0 ± 8.5	37.0* ± 8.7	40.1* ± 5.5

*$p < 0.05$.

Table 5. The effect on SOD activity of PGQ.

	Sodium chloride group	*Dopamine group* ($5\,mg{\cdot}kg^{-1}$)	*Low-dose drug group* ($1\,mg{\cdot}kg^{-1}$)	*Middle-dose drug group* ($2\,mg{\cdot}kg^{-1}$)	*High-dose drug group* ($4\,mg{\cdot}kg^{-1}$)
Baseline	486.3 ± 74.5	485.8 ± 47.4	506.0 ± 57.8	491.8 ± 81.0	486.3 ± 47.3
120 min	281.7 ± 39.9	369.5** ± 28.7	293.8 ± 52.3	320.3 ± 23.7	346.3* ± 30.8

*$p < 0.05$, **$p < 0.01$.

The new ocotillol-type saponin (**1**): white power, easily dissolved in water. mp 184–186°C, $[\alpha]_D^{20}$ + 27.8 (MeOH; c 0.72). IR (KBr) cm^{-1}: 3479, 2927, 1073. HR-MS (ESI): *m/z* 824.0212 $[M + Na]^+$ (calculated for $C_{42}H_{72}O_{14}$ + Na^+, 824.0236). ^1H NMR (500 MHz, C_5D_5N): 4.16 (1H, d, J = 10.0 Hz), 3.75 (1H, m), 3.27 (1H, m), 4.93 (1H, d, J = 8.0 Hz), 5.37 (1H, d, J = 7.5 Hz), 1.00, 0.83, 1.30, 1.30, 1.45, 1.28, 1.11, 0.91 (each 3H,s). For ^{13} C NMR data see table 1.

3.3 Biological activity

Haemorrhagic shock causes a variety of metabolic derangements. Recovery depends on the adequacy and speed of resuscitation. In this paper, thirty adult mongrel dogs weighing 14–25 kg were used. The dogs were fasted for 16 h, but were given water before the experiment [12]. They were premedicated with an intravenous injection of 30 mg pentobarbital sodium/kg. Anaesthesia was maintained after endotracheal intubation with thiopental sodium infusion (4–6 $mg \cdot kg^{-1} \cdot h^{-1}$) without respiratory depression. The animals were placed in the supine position, and the neck and the inguinal region were surgically prepped and draped. A catheter was insert into the trachea; the catheter, which was in the right carotid, was connected to pressure transducers through an AP-601G carrier wave amplifier for monitoring of arterial blood pressure. The needle electrodes were inserted into hypodermal skin to be monitored by electrocardiogram; the catheter in the right femoral vein was for the drug administration. The dogs were allowed to stabilise for at least 60 min after the surgical procedure, and physiological measurements (baseline) were obtained. Then, each haemodynamic index was recorded before shock. Blood was taken from the artery to measure blood gases; blood was taken from the vein to acquire serum to measure LPO [13] and SOD activities (SOD activities were assayed by a SOD kit [14]). Haemorrhagic hypotensive shock was induced by withdrawing blood from the left femoral vein into a reservoir until MAP stabilised at $<$5.33 kPa. The dogs were bled (37 $\pm$ 9 ml/kg) for 16 $\pm$ 5 min via the femoral venous catheter. A 30-min stabilisation period was allowed after induction of haemorrhagic hypotension (shock) to record each parameter of shock. The dogs were randomly assigned to one of five groups ($n = 6$ in each): (1) resuscitation with i.v. sodium chloride (0.9%); (2) resuscitation with i.v. dopamine (5 $mg \cdot kg^{-1}$); (3) resuscitation with i.v. new saponin solution (4 $mg \cdot kg^{-1}$); (4) resuscitation with i.v. new saponin solution (2 $mg \cdot kg^{-1}$); (5) resuscitation with i.v. new saponin solution (1 $mg \cdot kg^{-1}$). Each haemodynamic parameter was recorded after administration, and then after 5, 10, 20, 30, 40, 60, 90 and 120 min. Blood was obtained from the femoral artery to analyse blood gas parameters 60 min and 120 min after administration; venous blood was obtained after 120 min; the serum was separated and used to measure LPO and SOD activities. All the results were expressed as mean $\pm$ SD, and the measurements were evaluated by *t* test. Differences were considered significant at $p < 0.05$.

There were no significant differences between groups in initial MAP, prehaemorrhage weight, haemorrhage volume or haemorrhage time.

3.3.1 The effect on MAP. MAP in the low-dose group was significantly higher than in the sodium chloride group 20 and 30 min after administration ($p < 0.05$); MAP in the middle-dose group rose markedly compared with the sodium chloride group ($p < 0.05$) at 20 and 30 min after administration ($p < 0.05$); MAP in the high-dose group was significantly higher

than in the sodium chloride group ($p < 0.05$, $p < 0.01$, $p < 0.001$) at 120 min; MAP in the dopamine group was greater than that of the sodium chloride group for 5–20 min after administration ($p < 0.05$, $p < 0.01$ respectively) (table 2).

3.3.2 The effect on artery blood oxygen content. Compared with the sodium chloride group, the middle-dose and the dopamine groups' arterial blood oxygen contents were significantly higher ($p < 0.05$) (table 3).

3.3.3 The effect on LPO. Compared with sodium chloride group, LPO activities in the middle-dose and high-dose groups were both significantly lower ($p < 0.05$) 120 min after administration; the dopamine group's LPO activity was significantly lower ($p < 0.05$) 120 min after administration (table 4).

3.3.4 The effect on SOD. Compared with sodium chloride group, the SOD activity in the high-dose group and its rate of increase were both significantly higher ($p < 0.05$, $p < 0.01$) 120 min after administration; SOD activity in the dopamine group and rate of increase were both significantly higher ($p < 0.01$) 120 min after administration (table 5).

Acknowledgements

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