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Original Research Article

Investigation of hemorheological and analgesic properties of Qing-Nao tablets

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Abstract

Purpose: *To investigate the hemorheological and analgesic potentials of Qing-Nao tablet (QNT).*
Methods: *Sixty animals were divided into 6 groups (n = 10 for each group): normal, control, positive and 3 QNT-treated groups (QNT-H, QNT-M, QNT-L). Animals in normal and control groups received normal saline orally, while those in positive and QNT groups were orally administered either a suspension of the contents of Nao-luo-tong capsules or QNT. Hemorheological indices, including blood viscosity, plasma viscosity, activated partial thromboplastin time (APTT), thrombin time (TT), prothrombin time (PT) and fibrinogen (FIB), were determined. Acetic acid-induced writhing, tail tenderness and hot plate tests, as well as tests on microcirculation and balance/coordination were also carried out.*
Results: *Acute toxicity results showed that QNT is a safe drug. Whole blood and plasma viscosities of the 3 QNT-treated groups significantly decreased ($p < 0.05$). However, TT levels of the 3 QNT-treated animals significantly increased ($p < 0.05$), whereas APTT and FIB values decreased significantly ($p < 0.01$). Moreover, QNT significantly increased analgesia in three animal models ($p < 0.05$). QNT-H also significantly improved balance and coordination abilities of mice ($p < 0.05$).*
Conclusion: *These results demonstrate that QNT improves microcirculation and analgesia, and may be a source of promising candidate drugs for the treatment of brain concussion sequelae.*

Keywords: *Qing-Nao tablet, Hemorheological, Analgesic effects, Brain concussion sequelae, Microcirculation, Balance and coordination abilities*

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INTRODUCTION

Headache is probably the most common and troubling sequelae of concussion or severe traumatic head injury [1,2]. Concussion-induced headache is often of brief duration after the injury, and tends to resolve spontaneously within days or weeks. However, some patients may develop chronic headaches lasting for months or even years [2]. Presently, concussion-related headaches are usually managed with western medicines such as Oryzanol^R and Rotundine^R [3]. Unfortunately, prolonged use of orthodox medical treatment is associated with poor clinical outcomes as well as adverse reactions.

Qing-Nao tablet (QNT) is a traditional Chinese medicine preparation, which consists of seven Chinese herbal medicines (*Angelicae dahuricae* Radix, *Angelicae sinensis* Radix, *Chuanxiong rhizoma*, *Ramulus uncariae* Cum Uncis, Asari Radix Et Rhizoma, *Mentha haplocalyx* Herba,

and Longgu). In QNT, *Angelicae dahuricae* Radix has detumescence and analgesic effects. Clinically, QNT is commonly used to treat headache, dizziness, and brain concussion sequelae [3, 4]. In a study on treatment of 168 patients with brain injury syndrome, it was reported that QNT had significantly higher therapeutic effects and safety than the western medicine Oryzanol^{R} [5]. However, the possible mechanisms involved in this therapeutic effect have not been investigated. The present study was carried out to investigate the analgesic and rheological properties of QNT in rat and mice models.

EXPERIMENTAL

Animals

Adult male and female specific pathogen-free (SPF) mice (18 - 21g) were obtained from Shandong Lukang Co., Ltd (Certificate no. 37005400000030) (Jinan, China). Another set of adult male SPF rats (250-300 g) were obtained from Ji'nan Pengyue Experimental Animal Breeding Co. Ltd, Jinan, China (certificate no. 37009200001350). The animal protocols used were according to the declaration of Helsinki promulgated in 1964 as amended in 1996 [6], and were approved by the Animal Care and Use Committee of The 371 Hospital of Chinese People's Liberation Army {No. SYXK(Yu) 2015-005}.

Chemicals and reagents

Qing-Nao tablets (QNT) were produced by the 371 Hospital of Chinese People's Liberation Army (Batch No. 20141125, Xinxiang, China); normal saline was purchased from Zhengzhou Chemical Pharmaceutical Co., Ltd (Batch No. 150409, Zhengzhou, China), while Nao-luo-tong capsules were obtained from Henan Longdu Pharmaceutical Co. Ltd (Batch no. 150309, Zhoukou, China). Chloral hydrate was purchased from Tianjin Guangfu Fine Chemical Research Institution (Batch no. 2012707, Tianjin, China); adrenaline hydrochloride (Adr) injection was product of Grand Pharma (China) Co. Ltd (Batch No.150512, Wuhan, China); thrombin time (TT) assay kit (Batch No.121152), prothrombin time (PT) assay kit (Batch no. 105277), activated partial thromboplastin time (APTT) assay kit (Batch no. 112185, ellagic acid) and fibrinogen assay (FIB) Kit (Batch no. 132087) were purchased from Shanghai sun Biological Technology Co. (Shanghai, China). Yun-dong-ding capsule was obtained from Henan Longdu Pharmaceutical Co. Ltd (Batch no. 150309), Henan, China.

Acute toxicity test on QNT

The acute toxicity study of QNT was conducted by treating mice with maximum concentration and volume of drug. A total of 40 mice were divided into 2 groups (n = 20), with equal number of male and female, including normal group and QNT treated group. The QNT-treated mice were orally administered 0.375 g/ml QNT (40ml/kg body weight) three times a day, while the mice in normal group received normal saline (0.4 ml/10g). After 3 hours of drug administration, the mice were monitored for behavioral changes including autonomic activities, feeding and secretions. Drug administration and observation were carried out for 14 days. Thereafter, the mice were sacrificed, and gross changes in liver, heart, spleen, lung, kidney, stomach, intestines, and brain were observed.

Determination of effect of QNT on rats and mice with blood stasis

Sixty male rats were divided into 6 groups (n = 10): normal, control, positive and 3 QNT-treated groups (0.375, 0.75 and 1.5 g/kg). The rats in normal and model groups were administered orally with normal saline (10 ml/kg). The positive group and QNT-treated groups were orally administered suspension of Nao-luo-tong capsules (0.5 g/kg body weight), and QNT (0.375, 0.75 and 1.5g/kg), respectively. After 8 days of pretreatment, Adr (0. 8mL/kg) was injected subcutaneously into rats (except the normal group) twice a day. Then the rats were placed in ice-cold water for 4min for development of blood stasis. After a 24-h fast, rats in the 6 groups were orally administered their respective drugs. Thirty minutes later, blood samples were collected from the carotid artery under anesthesia (chloral hydrate, 45 mg/kg, intraperitoneal injection) into 3.8 % sodium citrate sample vials. The hemorheological indices of blood samples were determined. The parameters assayed were whole blood viscosity (ηb), plasma viscosity (ηp), APTT, TT, PT, and FIB.

The study was also carried out in mice. Sixty mice of both sexes were divided into 6 groups (n = 10): normal, control, positive and 3 QNT treated groups (2.24, 1.12 and 0.56 g/kg body weight). The mice in normal and model groups were treated orally with normal saline (0.1ml/10g). The positive and QNT-treated mice were orally administered a suspension of Nao-luo-tong capsules (0.75 g/kg body weight), and QNT (0.375, 0.75 and 1.5 g/kg body weight, respectively). After 15 days of continuous drug treatment, all groups (except the normal group)

were administrated dexamethasone (0.8 mg/kg) by intramuscular injection once a day for 15 days. Then blood samples were collected from the eyeballs into 3.8% sodium citrate sample vials for determination of whole blood viscosity (ηb), plasma viscosity (ηp), APTT, TT, PT, and FIB.

Determination of whole blood viscosity, plasma viscosity, APTT, TT, PT, and FIB

Blood samples were centrifuged at 3000 rpm for 10 min to obtain plasma samples, and then whole blood viscosity and plasma viscosity was determined using LG-R-80 blood viscosity instrument (Beijing Steellex Science Instrument Co., Beijing, China). In addition, plasma APTT, TT, PT, and FIB were determined using commercial kits for APTT, TT, PT, and FIB according to the manufactures' instructions, and with LG-PABER platelet aggregation and coagulation factor analyzer (Beijing Steellex Science Instrument Co., Beijing, China), respectively.

Evaluation of effect of QNT on mice with auricular microcirculation disturbance induced by adrenaline

Fifty mice of both sexes were divided into 5 groups (n = 10): normal, positive and 3 QNT treated groups (2.24, 1.12 and 0.56 g/kg). The mice in normal group received normal saline orally (10 ml/kg). The positive and QNT-treated mice were orally administered a suspension of Nao-luo-tong capsules (0.75g/kg) and QNT (2.24, 1.12 and 0.56 g/kg), respectively.

After 10 days of this pretreatment, all groups (except the normal group) were administrated 5 % chloral hydrate (0.03 mL/10g) anesthesia by intraperitoneal injection. Then the mice were fixed in observation platform, and a little cedar oil was put in the ear of each mouse. The diameter of fine auricular artery (A) and vein (V), blood flow velocity and capillary opening number of mice were measured using automatic image analyzer. Then the mice were administered adrenaline hydrochloride injection (10 mg/kg body weight) through the tail vein. After 2 min, the diameter of fine auricular artery (A) and vein (V); blood flow velocity and extent of capillary opening of mice were measured again.

Analgesic effect of QNT: acetic acid-induced writhing test in mice

Fifty mice of both sexes were divided into 5 groups (n = 10): control group, positive treated group and 3 QNT treated groups (2.24, 1.12and 0.56 g/kg body weight). The mice in control group received normal saline (0.1 ml/10g). The positive and QNT treated mice were orally administered a suspension of Yun-tong-ding capsule (0.9 g/kg body weight), and QNT (2.24, 1.12 and 0.56 g/kg body weight), respectively. After 3 days of pretreatment, all groups (except the cormal group) received intraperitoneal injection of 0.5% acetic acid solution (0.1 ml/10g). Then the incubation period before the first writhe, and the no. of writhes (WN) within 10 min were recorded. Analgesia was calculated using Eq 1.

$$\text{Analgesia (\%)} = \{(WNc - WNt)/WNc\}100 \quad \ldots. (1)$$

where WNc and WNt are the number of writhes for control and treatment groups, respectively.

Tail tenderness experiment

The groupings and drug administration were the same as described earlier. Before the mice were treated with the drug, their basic pain thresholds were measured by using ZH-LUO/B mice tail pain instrument (Yi-lian Medicine Instrument Development Co., Ltd, Shanghai, China). After 7 days, the pain threshold (PT) of mice in the 5 groups was measured again. Analgesic level was calculated as in Eq 2.

$$\text{Analgesia (\%)} = \{(PTt - PTb)/PTb\}100 \quad \ldots\ldots. (2)$$

where PTb and PTt are the pain threshold before and after treatment, respectively.

The increase in value of pain threshold was negative, which could be considered to be zero [7,8].

Hot plate test

Female mice with normal pain threshold (5 - 30 s) grouping and administrations were the same as described earlier. Pain models of mice were made by using RB-200 hot plate (Chengdu Taimeng Software Co. Ltd, Chengdu, China) at 55.0 ± 0.5 °C. The pain thresholds of mice were defined as the time taken before the mice did the first jump after placing the mice on the hot plate. The mice were treated with drugs for 3 days. Pain thresholds were determined again after 30, 60, and 120 min from the last administration. If a mouse showed no response at 60 s, it was removed so as to avoid burn injury, and its pain threshold was taken as 60 s. Analgesia (%) was calculated as in Eq 2.

The increase in value of pain threshold was negative, which could be considered to be zero

[7,8].

Determination of effect of QNT on balance and coordination of mice

Mice grouping and drug administration were the same as described earlier. After 10 days of treatment, the mice were put on ZB-200 fatigue rotating rod instrument (30 rev/ min). The time taken before mice fell off from the rotating rod was recorded repeatedly 3 times. The longest time and sum of the 3 times were used as observation indices.

Statistical analysis

Data are presented as mean ± SD, and statistical analysis was conducted using Student's t-test with SPSS 17.0 (SPSS Inc, USA). Differences were considered significant at $p < 0.05$.

RESULTS

Acute toxicity of QNT

There were no obvious gross changes in the normal group. Spontaneous activity of mice decreased initially, but returned to normal after 2 h. Feed intake was not affected, and there were no obvious changes in liver, heart, spleen, and other organs in normal and QNT-treated groups of mice. No anomalies were evident in organ indices, except renal index. These results from acute toxicity demonstrated that QNT was of very low oral toxicity, and may be considered a safe drug for clinical applications.

Effect of QNT on blood stasis in rats and mice

As shown in Table 1 and Table 2, whole blood viscosity of control mice significantly increased compared to the normal mice ($p < 0.01$). Whole blood viscosity (low and middle shear, $p < 0.01$; high shear, $p < 0.05$) and plasma viscosity ($p < 0.01$) of positive group rats were significantly lower than corresponding values for control rats. QNT (1.5, 0.75 and 0.375 g/kg) significantly reduced whole blood viscosity ($p < 0.05$) and plasma viscosity ($p < 0.05$) of rats when compared with control rats. However, the decreases in whole blood viscosity of mice in the QTN-L group were not statistically significant.

As shown in Table 3, TT and PT values in control group were decreased whereas the APTT and FIB values significantly increased relative to normal rats. The TT levels of positive and QNT-L-treated rats significantly increased when compared with control rats ($p < 0.05$). In the 3 QNT groups (1.5, 0.75 and 0.375 g/kg), TT of rats was prolonged whereas APTT ($p < 0.01$) was shortened, while FIB was significantly higher when compared with control ($p < 0.01$).

Table 1: Effect of QNT on whole blood viscosity and plasma viscosity of rats with blood stasis (n = 10)

Group	Dose (g/kg)	Whole blood viscosity (mPa·s)			Plasma viscosity (mPa·s)
		Low shear rates ($10s^{-1}$)	*Middle shear rates ($60s^{-1}$)*	*High shear rates $150s^{-1}$*	
Normal	----	11.07±1.92**	6.46±0.88**	5.21±0.54**	1.57±0.34*
Control	----	20.74±1.42	9.41±0.62	6.94±0.45	2.04±0.27
Positive	0.5	16.79±1.34**	7.96±0.63**	6.29±0.32*	1.54±0.08**
QNT-H	1.5	17.76±2.22**	8.11±0.86*	6.23±0.47*	1.58±0.17**
QNT-M	0.75	17.56±1.67**	8.26±0.51**	6.22±0.71	1.62±0.05**
QNT-L	0.375	18.50±2.12*	8.91±0.67	6.76±0.45	1.64±0.09*

*QNT-H, QNT-M, and QNT-L mean high, middle and low doses of QNT. Data are expressed as mean ± SD (n = 10), * p < 0.05, ** p < 0.01, compared with the control group*

Table 2: Effect of QNT on whole blood viscosity of mice with blood stasis (n = 10)

Group	Dose (g/kg)	Whole blood viscosity (mPa·s)		
		Low shear $10s^{-1}$	Middle shear $60s^{-1}$	High shear $150s^{-1}$
Normal	----	5.77±0.38	3.89±0.38	3.35±0.24
Control	----	8.81±0.4**	5.10±0.38**	4.41±0.38**
Positive	0.75	8.11±0.37**	4.63±0.34**	3.93±0.31**
QNT-H	2.24	8.37±0.38*	4.66±0.26**	3.95±0.32**
QNT-M	1.12	8.46±0.32*	4.77±0.29*	4.08±0.32*
QNT-L	0.56	8.78±0.32	4.99±0.38	4.30±0.41

*QNT-H, QNT-M, and QNT-L means high, middle and low doses of QNT. Data are expressed as Mean ± SD (n = 10), * p < 0.05, ** p < 0.01, compared with the control group*

Table 3: Effect of QNT on APTT, TT, PT, and FIB of rats with blood stasis (n = 10)

Group	Dose (g/kg)	TT (s^{-1})	PT (s^{-1})	APTT (s^{-1})	FIB ($mg·\mu L^{-1}$)
Normal	----	22.76±1.09**	16.44±0.70**	51.07±7.08**	198.2±16.75**
Control	----	19.61±0.65	12.82±0.31	64.31±9.50	259±15.42
Positive	0.5	20.88±0.93*	12.94±0.43	55.32±6.81*	268±18.74
QNT-H	1.5	22.13±1.36**	13.08±0.47	49.89±10.19**	196±23.55**
QNT-M	0.75	20.71±1.22	12.6±0.37	48.54±9.04**	186.5±18.77**
QNT-L	0.375	20.90±1.04*	12.62±0.46	48.26±10.07**	183±22.85**

*QNT-H, QNT-M, and QNT-L means high, middle and low doses of QNT. Data are expressed as Mean ± SD (n = 10), * p < 0.05, ** p < 0.01, compared with the control group*

Effect of QNT on mice with auricular microcirculation disturbance induced by adrenaline

The results of the effect of QNT on auricular microcirculation disturbance induced by adrenaline are shown in Table 4 and Table 5. QNT-H (2.24 g/kg, $p < 0.05$), and Nao-luo-tong capsules significantly inhibited vasoconstriction of fine auricular vein of mice, compared with normal mice ($p < 0.01$). In addition, the vasoconstriction of fine auricular artery in the positive group, and QNT-treated groups were significantly inhibited when compared with normal mice ($p < 0.01$). As presented in Table 5, QNT ($p < 0.01$) and Nao-luo-tong significantly increased extent of capillary opening in mice with auricular microcirculation disturbance induced by adrenaline. The results demonstrate that QNT could significantly improve microcirculation and blood flow of mice with auricular microcirculation disturbance induced by adrenaline.

Analgesic effect of QNT

The results of acetic acid-induced writhing test, tail tenderness test and hot plate test are presented in Table 6, Table 7 and Table 8, respectively. As shown in Table 6, QNT (2.24 and 1.12 g/kg, $p < 0.01$; 0.56 g/kg, $p < 0.05$), and Yun-tong-ding ($p < 0.01$) significantly increased the latency of writhing, and also significantly decreased writhing times of mice within 10 min when compared with mice in the normal group. Pain thresholds of mice in the tail tenderness test was also significantly increased in the QNT groups (2.24, 1.12 and 0.56 g/kg), and in the positive treated groups, relative to normal mice

Table 4: Effects of QNT on the diameter of fine auricular artery (A) and vein (V) of mice with auricular microcirculation disturbance induced by adrenaline (n = 10)

Group	Dose (g/kg)	Diameter of fine auricular vein before adrenaline injection (μm)	Diameter of fine auricular vein after adrenaline injection(μm)	Diameter of fine auricular artery before adrenaline injection (μm)	Diameter of fine auricular artery after adrenaline injection (μm)
Normal	----	77.80±12.03	49.69±5.31	45.41±7.34	30.24±2.71
Positive	0.75	74.19±16.30	60.90±5.10**	46.29±7.84	35.41±2.09**
QNT-H	2.24	74.23±7.95	57.37±4.17*	43.62±5.83	36.69±3.29**
QNT-M	1.12	76.96±13.56	56.89±7.65	43.87±9.41	36.76±4.04**
QNT-L	0.56	75.76±10.56	52.99±4.74	44.37±8.22	36.80±3.00**

*QNT-H, QNT-M, and QNT-L mean high, middle and low doses of QNT. Data are expressed as Mean ± SD (n = 10), * p < 0.05, ** p < 0.01, compared with the control group*

Table 5: Effect of QNT on capillary opening amount of mice with auricular microcirculation disturbance induced by adrenaline (n = 10)

Group	n	Dose (g/kg)	Capillary opening of mice before adrenaline injection	Capillary opening of mice after adrenaline injection
Normal	10	----	4.4±0.84	2.7±0.48
Positive	10	0.75	4.5±0.71	3.7±0.67**
QNT-H	10	2.24	4.7±0.68	3.6±0.70*
QNT-M	10	1.12	4.6±0.70	3.6±0.52**
QNT-L	10	0.56	4.5±0.53	3.1±0.57

*QNT-H, QNT-M, and QNT-L mean high, middle and low doses of QNT. Data are expressed as Mean ± SD (n = 10), * p < 0.05, ** p < 0.01, compared with the control group*

(p < 0.01; Table 7). In addition, QNT (2.24 and 1.12 g/kg) and Yun-tong-ding significantly improved pain thresholds in the hot plate test 30 min post-drug treatment, when compared with mice in normal group (p < 0.01; Table 8). After 60 min of treatment, QNT (2.24 g/kg, p < 0.01; 1.12 g/kg, p <0.05), and Yun-tong-ding (p < 0.01) also significantly increased pain thresholds of mice, when compared with mice in normal group. In addition, after 120 min, pain thresholds of mice in the three QNT groups (2.24 and 1.12 g/kg, p < 0.01; 0.56 g/kg, p < 0.05) and positive group (p <

Table 6: Acetic acid-induced writhing test results (n =10)

Group	Dose (g/kg)	Incubation period (min)	Writhing times within 10min	Analgesic rate (%)
Normal	----	3.41±0.57	33.6±4.14	----
Positive	0.9	5.01±0.56**	19.9±4.07**	40.77
QNT-H	2.24	4.82±0.71**	21.7±5.10**	35.42
QNT-M	1.12	4.48±0.60**	24.3±4.67**	27.68
QNT-L	0.56	4.17±0.66*	27.6±5.46*	17.86

*QNT-H, QNT-M, and QNT-L mean high, middle and low doses of QNT. Data are expressed as Mean ± SD (n = 10), *p < 0.05, **p < 0.01, compared with the control group*

Table 7: Tail tenderness test results (n = 10)

Group	Dose (g/kg)	Basic pain thresholds (g)	Pain thresholds after drug treatment (g)	Analgesic rate (%)
Normal	----	270.39±25.48	342.21±22.95 (71.82±15.98)	7.58
Positive	0.9	267.35±20.67	532.46±36.76 (265.11±25.63)**	99.64
QNT-H	2.24	266.43±21.00	528.99±24.55 (262.56±28.88)**	99.63
QNT-M	1.12	264.68±32.27	486.04±30.17 (221.36±14.77)**	85.08
QNT-L	0.56	267.70±30.13	459.44±36.60 (191.74±28.92)**	72.93

*QNT-H, QNT-M, and QNT-L means high, middle and low doses of QNT. Data are expressed as Mean ± SD (n = 10), * p < 0.05, ** p < 0.01, compared with the control group. The data in brackets is the increase in value of pain threshold*

Table 8: Hot plate test results (n = 10)

Group	Dose (g/kg)	Basic pain thresholds	Pain thresholds at different time after drug treated		
			30 min	*60 min*	*120 min*
Normal	----	14.9±1.11	20.37±1.20 (5.50±1.01)	22.15±1.28 (7.28±1.54)	23.14±1.64 (8.27±2.26)
Positive	0.9	14.5±1.34	21.58±1.22 (7.07±1.16)**	23.96±1.95 (9.45±1.53)**	26.26±1.92 (11.75±1.77)**
QNT-H	2.24	14.8±1.21	22.03±0.91 (7.22±1.62)	23.04±1.07 (8.23±1.44)	25.95±1.86 (11.14±2.76)*
QNT-M	1.12	15.0±1.75	23.16±1.83 (8.16±1.33)**	23.76±1.43 (8.77±1.55)*	26.81±1.88 (11.82±2.65)**
QNT-L	0.56	14.6±1.77	23.41±1.94 (8.86±1.85)**	24.08±2.02 (9.56±1.54)**	26.67±3.12 (12.12±2.85)**

*QNT-H, QNT-M, and QNT-L mean high, middle and low doses of QNT. Data are expressed as Mean ± SD (n = 10), *p < 0.05, **p < 0.01, compared with the control group. The data in brackets is the increase in value of pain threshold*

Table 9: Effect of QNT on balance and coordination abilities of mice (n = 10)

Group	Dose (g/kg)	1st (s)	2nd (s)	3rd (s)	Longest time (s)	Sum of 3 time (s)
Normal	----	25.9±6.77	34.1±8.99	40.1±6.03	41.7±5.98	100.1±16.85
Positive	0.9	33.6±5.10	42.0±8.71	47.9±7.84	48.3±7.60*	123.5±19.16**
QNT-H	2.24	30.7±7.04	41.2±6.12	44.3±8.39	47.2±5.37*	116.2±15.63*
QNT-M	1.12	27.5±7.56	36±10.52	40.7±5.96	43.5±7.82	104.2±16.75
QNT-L	0.56	26.0±6.22	33.3±8.72	39.1±8.90	42.8±6.61	98.4±13.61

*QNT-H, QNT-M, and QNT-L mean high, middle and low doses of QNT. Data are expressed as Mean ± SD (n = 10), * p < 0.05, ** p < 0.01, compared with the control group*

0.01) were significantly higher than corresponding values for mice in the normal mice group. These results clearly demonstrate that QNT exerts potent analgesic effect.

Effect of QNT on balance and coordination abilities of mice

Results of effects of QNT on balance and coordination of mice are presented in Table 9. From these results, it is clear that QNT-H at 2.24 g/kg ($p < 0.05$); and Yun-tong-ding capsule ($p < 0.05$, $p < 0.01$) significantly prolonged the time taken before mice fell off the rotating rod, when compared with mice in the normal group. This shows that QNT can significantly improve balance and coordination ability of mice.

DISCUSSION

In the present investigation, the effect of QNT on microcirculation and analgesia were investigated using standard procedures. Studies have shown that Traditional Chinese Medicines (TCM) are associated with low side-effects, and contain potential drugs that could treat various diseases, particularly those that defy modern synthetic drugs [9,10]. The results of acute toxicity test clearly showed that QNT has very low oral toxicity. This implies that it is safe for clinical applications.

Brain injury syndrome is classified under “Headache” in TCM system. It is thought to be caused by *Qi* and blood stasis / disorder. Brain injury disorder results in a series of symptoms, such as dizziness, headache, nausea and tinnitus. Therefore, in TCM system, brain injury syndrome is commonly treated by using Chinese herbal medicines that are effective for cleansing the blood, treating stasis and effecting analgesia [12,13]. Blood stasis refers to the patho-physiological state of ‘blood stagnation’ characterized by vasoconstriction and delayed blood flow, which are the most common clinical features of brain injury syndromes [11]. Concussion-induced headache usually results in changes in microcirculation in the head, as well as blood stasis. The changes in microcirculation may be reversed by down-regulating the expression of D-Dimer [14].

QNT consists of 7 Chinese herbal medicines. It has been reported that water extracts of *Angelicae sinensis* Radix and *Chuanxiong rhizoma* decreased hemorheological indices, prolonged PT and APTT, and significantly reduced levels of FIB [15].Thus, in a QNT prescription, *Angelicae sinensis* Radix and *Chuanxiong frhizoma* normalize blood by resolving blood stasis. Results obtained in the present study also showed that QNT improved microcirculation. *Angelicae dahuricae* and the other Chinese herbal medicine components of QNT have detumescent and analgesic effects [5]. A series of analgesic studies on QNT demonstrated that it possesses significant analgesic effects [5]. Therefore, QNT is capable of exerting analgesic effects on headache induced by a concussion. These results strongly suggest that QNT could be a promising source of candidate drug for the treatment of brain concussion sequelae.

CONCLUSION

The findings of this study demonstrate that QNT possesses significant microcirculation-improving properties, as well as balance and coordination-enhancing analgesic properties. Thus, it may be a promising source of new candidate drugs for the treatment of brain concussion sequelae, which is usually characterized by headache and dizziness.

DECLARATIONS

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Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them.

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