

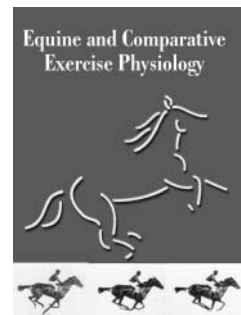
The effect of herbal supplementation on the severity of exercise-induced pulmonary haemorrhage

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Abstract

Exercise-induced pulmonary haemorrhage (EIPH) is a serious condition that affects the health and possibly the performance of all racehorses. However, only two treatments, furosemide and the Flair™ equine nasal strip, both of which reduce capillary transmural pressure, have been successful in reducing EIPH. Alternatively, transient impairment of platelet function and coagulation during exercise has been considered an additional contributor to EIPH. Consequently, herbal formulations designed to enhance platelet function, and hence coagulation, are hypothesized to reduce EIPH. To investigate the validity of this hypothesis, five Thoroughbred horses completed three maximal incremental exercise tests on a 10% inclined treadmill in a randomized cross-over design experiment. Treatments included twice daily oral administration (for 3 days) of a placebo (PL; cornstarch) and two herbal formulas, *Yunnan Paiyao* (YP) or *Single Immortal* (SI). Blood samples for coagulation profiles, complete blood counts and biochemistry profiles were collected before each exercise test. During each test, pulmonary arterial pressure, oxygen uptake, arterial blood gases, plasma lactate and time-to-fatigue were measured. Severity of EIPH was quantified via bronchoalveolar lavage (BAL) at 30–60 min post-exercise. The herbal formulations were not effective in decreasing EIPH ($\times 10^6$ red blood cells ml⁻¹ BAL fluid: PL, 27.1 ± 11.6 ; YP, 33.2 ± 23.4 ; SI, 35.3 ± 15.4 , $P > 0.05$) or in changing any of the other variables measured with the exception of time-to-fatigue, which was slightly but significantly prolonged by *Single Immortal* compared with placebo and *Yunnan Paiyao* (PL, 670 ± 9.6 s; YP, 665 ± 5.5 s; SI, 685 ± 7.9 s, $P < 0.05$). Thus, these results do not support the use of these herbal formulations in the prevention of EIPH.

Keywords: exercise-induced pulmonary haemorrhage; coagulation; pulmonary arterial pressure; bronchoalveolar lavage; stress failure of the pulmonary capillaries; herbal medicine

Introduction

Exercise-induced pulmonary haemorrhage (EIPH) is a ubiquitous phenomenon in equine athletes exercising at or near maximal effort^{1,2}. Since the condition was first documented in 16th-century racehorses³, research efforts have focused on mitigation of the haemorrhage. The development of effective treatments that will prevent and/or diminish the severity of EIPH has become paramount in importance owing to the potentially serious consequences of recurrent pulmonary haemorrhage on the health and welfare of performance horses. These negative consequences and cumulative effects of

EIPH include inflammation, scarring⁴⁻⁷ and (possibly) decreased performance⁸⁻¹⁰. However, only two treatments to date have demonstrated scientific efficacy in the amelioration of EIPH. Furosemide, which significantly lowers pulmonary arterial pressures¹¹, a primary factor in the initiation of EIPH¹², reduces EIPH by 50–90% in strenuously exercising horses¹³⁻¹⁵. Unfortunately, the use of furosemide is problematic, in part because its effects are variable but also because of its ability to enhance performance, as well as mask the detection of illegal drugs^{16,17}. The Flair™ equine nasal strip decreases EIPH by 30–50%^{13-15,18,19} as a result

of maintaining nasal patency and reducing upper airway resistance during inspiration²⁰, thereby reducing the work of breathing and, ultimately, lowering the capillary transmural pressure. Unfortunately, neither of these treatments alone or in combination has been able to completely eliminate EIPH¹⁵, and both are banned from use in many racing jurisdictions.

Early studies^{21–26} investigating a proposed relationship between a coagulation anomaly in exercising horses and the potentiation of EIPH suggested that horses may have decreased clotting ability during exercise, thus increasing the amount of haemorrhage observed consequent to an impaired ability to rapidly plug breaks that occur in the blood-gas barrier during strenuous exercise. In light of these data, herbal formulations have been analysed and anecdotally reported to be helpful in addressing this problem, and are in widespread use on the racetrack. However, controversy arises since the only proof of benefit to date has been clinical impressions of efficacy, and no scientific evidence exists to support the use of herbal formulations for the prevention or reduction of EIPH. In fact, the most recent literature refutes the validity of inferences made from the earlier studies (suggesting that platelet function defects may be present in exercising horses) as a result of the discovery that the anticoagulant used may itself inhibit platelet function^{27,28}. Therefore, it seems unlikely that shortened coagulation and enhanced platelet function would reduce the severity of EIPH.

The purpose of the present investigation was to determine the effectiveness of two herbal formulations (*Yunnan Paiyao* and *Single Immortal*) on coagulation and EIPH in the horse. The individual ingredients in these formulations have been shown to enhance coagulation by decreasing the bleeding time in other species (rats and rabbits)^{29–31}, and *Single Immortal* has anecdotally been reported to be effective in reducing EIPH in horses³². We hypothesized that EIPH would not be reduced following maximal exercise when evaluated by bronchoalveolar lavage (BAL), whether or not coagulation variables or bleeding times were shortened as a result of treatment with the herbal formulations.

Materials and methods

Animals

Five Thoroughbred horses, aged 5–14 years and weighing 470–600 kg with a documented history of EIPH, were used in this study. The animals were housed on a dry lot with loafing sheds and free access to water and salt. They were fed alfalfa and free-choice grass hay, as well as concentrate (Strategy; Purina Mills Inc., St. Louis, MO) twice daily. They were dewormed at 3-month intervals, rotating ivermectin

with oxibendazole, and vaccinated against eastern and western encephalomyelitis, tetanus, equine influenza, West Nile virus, rabies and equine herpes I. The horses were trained on a high-speed treadmill (SATO Inc., Uppsala, Sweden) three days per week and had food, but not water, withdrawn for at least 2 h before experimentation. All procedures were approved by the Kansas State University Animal Care and Use Committee.

Treatments

Treatment order was randomized in a cross-over design. The investigators were blinded to which treatment the experimental subjects had received. Cornstarch (5 tablespoons) was administered as the placebo by mouth twice daily for 3 days prior to the exercise test, as well as on the morning of the maximal exercise test. A patented Chinese herbal formula called *Yunnan Paiyao* (Mayway Corporation, Oakland, CA) and another herbal formulation called *Single Immortal* (Jing-Tang Herbal Company, Reddick, FL) were tested. The doses utilized were those either recommended by the manufacturer (*Single Immortal*) or considered effective from widespread administration on the racetrack (*Yunnan Paiyao*). Two full weeks were allowed for wash-out time between treatments. *Yunnan Paiyao* was administered to the horses at a dose of 4 g of powder by mouth twice daily for 3 days before the trial, and on the morning of the maximal exercise test. *Single Immortal* was administered for 3 days before exercise testing at a dose of 50 g of powder by mouth twice daily, including the morning of the maximal exercise test³². Blood samples were obtained by jugular venepuncture for complete blood counts (CBC), biochemistry profiles, coagulation assays (prothrombin time and partial thromboplastin time) and platelet counts immediately before all maximal exercise tests. Control blood samples (from normal, healthy horses not on the experiment) were also submitted with the experimental coagulation assay samples.

Animal preparation

Prior to the exercise test, each horse had two 7-F introducer catheters placed in the right jugular vein and one 18-gauge, 2 in catheter (Safelet; NIPRO Medical Corporation, Miami, FL) placed in either a previously elevated left carotid artery or the transverse facial artery (one horse). These procedures were performed under local anaesthesia (2% lidocaine) using aseptic techniques. A carotid arterial cannula (polyethylene; 1.6 mm inner diameter and 3.2 mm outer diameter) was connected to the arterial catheter to facilitate withdrawal of arterial blood. A 7-F microtipped pressure transducer (Millar Instruments, Inc., Houston, TX) was placed into the pulmonary artery through one of the 7-F introducer catheters, approximately

8 cm past the pulmonic valve, to monitor pulmonary arterial pressure. A Fourier analysis of the pulmonary arterial pressure waveform was performed and the numerical value of the first peak (~ 2 Hz) was multiplied by 60 cycles s^{-1} to obtain the respiratory rate³³, since this peak has been shown to correspond to the fundamental frequency for this variable. The location of the pressure transducer and the thermistor was verified by cardiac waveform evaluation via a data analysis system (DATAQ, Akron, OH) and viewed on a monitor. The Millar pressure transducer was calibrated prior to and immediately following each experimental run in 50 mmHg increments (range 0–200 mmHg) with a mercury manometer. A thermistor (Columbus Instruments, Columbus, OH) was advanced through the other 7-F introducer catheter into the right pulmonary artery to measure pulmonary arterial temperature, allowing for temperature correction of blood gases and pH³⁴. The thermistor was calibrated using a thermocouple thermometer (BAT-10; Physiotemp, Clifton, NJ). Oxygen consumption was measured with a bias flow system as previously described by Kindig *et al.*³⁵. Heart rate was determined with a heart rate monitor (Polar, Mill Valley, CA).

Maximal exercise test

Each horse completed one maximal exercise test on the inclined treadmill (10% incline) after each of the following treatments: placebo, *Yunnan Paiyao* and *Single Immortal*. After resting measurements were made with the horses standing quietly on the treadmill, each horse was warmed up at 3 m s^{-1} for 2 min. The horses then performed an incremental exercise test (speed increasing by 1 m s^{-1} per minute) beginning at 4 m s^{-1} to volitional fatigue, then recovered at a trot (3 m s^{-1} for 4 min). Cardiorespiratory measurements (heart rate, pulmonary arterial pressure, oxygen uptake ($\dot{V}O_2$) and carbon dioxide production ($\dot{V}CO_2$)) were collected continuously throughout exercise and cool-down, and arterial blood samples were collected during the last 10 s at each speed, as well as during recovery at 2 and 4 min after maximal exercise. BAL was performed at 30–60 min after the exercise test, to quantitate EIPH as described below^{2,15}.

Blood analysis

Following anaerobic withdrawal (into plastic, heparinized syringes), blood samples were placed immediately on ice. Within 1–2 h of the experiment, arterial blood gases were quantified by means of blood gas analysis (Nova Stat M; Nova Biomedical, Waltham, MA) and corrected to the horse's pulmonary arterial blood temperature³⁴. The blood gas analyser was calibrated before running the samples according to the manufacturer's standards.

Bronchoalveolar lavage

The horses were tranquilized using detomidine hydrochloride (Dormosedan[®], Pfizer Animal Health, Exton, PA; $5\text{--}10 \mu\text{g kg}^{-1}$ intravenously) and butorphanol tartrate (Torbugesic[®], Fort Dodge Animal Health, Fort Dodge, IA; $5\text{--}10 \mu\text{g kg}^{-1}$ intravenously) to facilitate BAL and to quantify the severity of EIPH at 30–60 min post-exercise^{2,15}. A Bivona tube (Bivona Medical Technology, Gary IN; 3 m long, 10 mm in diameter) with an inflatable cuff was introduced into the right naris through the ventral meatus and into the lung until wedged in a subsegmental bronchus of the dorsal caudal portion of the lung³⁶. The Bivona tube with a cuff created a seal within the airway, which ensured lavage of the distal airway and maximized recovery of lavage fluid. A total of 300 ml (in 50 ml aliquots) of 0.9% physiological saline was infused. After approximately two breaths, the fluid (a percentage of the entire 300 ml) was aspirated with gentle suction. Fluid recovery averaged approximately 60% of instilled volume; no significant differences in recovery existed between trials. The BAL fluid was centrifuged (Beckman TJ-6; Beckman Instruments, Inc., Palo Alto, CA), the supernatant decanted, and the pellet was re-suspended in 0.9% saline³⁷. Centrifugation, washing and re-suspending BAL fluid prior to cell counts results in no significant difference in total cell counts³⁷ and was utilized for the following reasons:

- to reduce mucus and debris that interfere with counting; and
- to achieve a similar final concentration of red blood cells (RBCs) across runs and horses to avoid errors in counting due to widely differing RBC concentrations.

The amount of saline used for re-suspension ranged from 10 to 200 ml, depending on the severity of EIPH, to maintain a relatively similar RBC-to-saline solution ratio (i.e. lavage fluid haematocrit). RBCs and total nucleated cells (TNCs) were counted using a haemocytometer (No. 02-671-5; Fisher Scientific, Pittsburg, PA) and a microscope (Nikon Instrument Group, Inc., Garden City, NY). Data are presented as RBCs and TNCs per ml of recovered BAL fluid minus tube dead space (17 ml). We consider this technique to be valid and reliable for evaluating treatment effectiveness since our laboratory has demonstrated that under controlled laboratory conditions in which horses run identical protocols, the recovery of BAL fluid and the number of RBCs per ml of lavage fluid are highly reproducible between runs (i.e. coefficient of variation $\sim 5\%$)¹⁵. Resting/control BAL samples were taken from the horses under light-moderate training with no strenuous high-intensity exercise performed within 10 days, and evaluated for RBCs and TNCs at the initiation of the study.

Statistical analysis

A one-way analysis of variance (ANOVA) for repeated measures was utilized to determine if differences existed between treatment variables measured across experimental conditions, with the exception of platelet numbers and $\dot{V}O_{2\max}$. These latter variables were normally distributed (Kolmogorov-Smirnov test for normality) but did not have equal variances, and were therefore analysed with one-way repeated-measures ANOVA on ranks. If significance was revealed with ANOVA, a Student-Newman-Keuls *post hoc* test was used to determine where the differences were located. Pre- and post-test variables (i.e. cutaneous bleeding times) were examined using a paired *t*-test. Statistical significance was accepted at the $P < 0.05$ level.

Results

Neither RBCs ml^{-1} BAL fluid (Fig. 1) nor white blood cells ml^{-1} BAL fluid (Fig. 2) were altered after treatment with *Yunnan Paiyao* (YP) or *Single Immortal* (SI). However, treatment with *Single Immortal* increased the time-to-fatigue by a small but significant amount over both *Yunnan Paiyao* and placebo (PL) exercise tests (PL, 670 ± 10 s; YP, 665 ± 6 s; SI, 685 ± 8 s, $P < 0.05$; Table 1). None of the other variables measured at maximal exercise (Table 1), including cardiorespiratory variables (heart rate, pulmonary arterial pressure, $\dot{V}O_{2\max}$ and $\dot{V}CO_{2\max}$, arterial blood gases, plasma lactate, acid-base variables or haematological variables), were altered with treatment. Of the original five horses, one was dropped from analysis of $\dot{V}O_{2\max}$ and $\dot{V}CO_{2\max}$ because of technical difficulties during data collection. Coagulation variables including platelet numbers, fibrinogen, partial thromboplastin time and prothrombin time were not altered

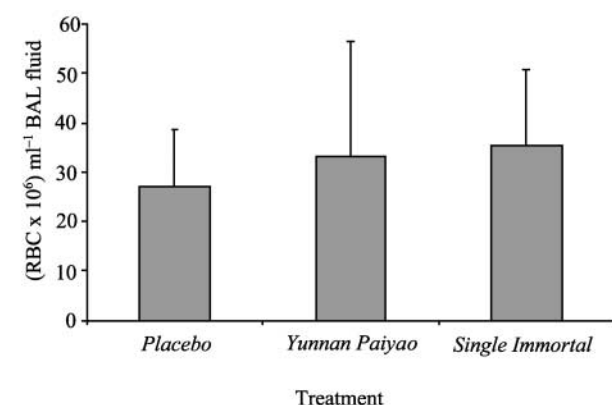


Fig. 1 Severity of exercise-induced pulmonary haemorrhage following maximal exercise ($n = 5$) as determined from the concentration of red blood cells (RBCs) in bronchoalveolar lavage (BAL) fluid after treatment with placebo, *Yunnan Paiyao* and *Single Immortal*. There were no significant differences between conditions ($P > 0.05$)

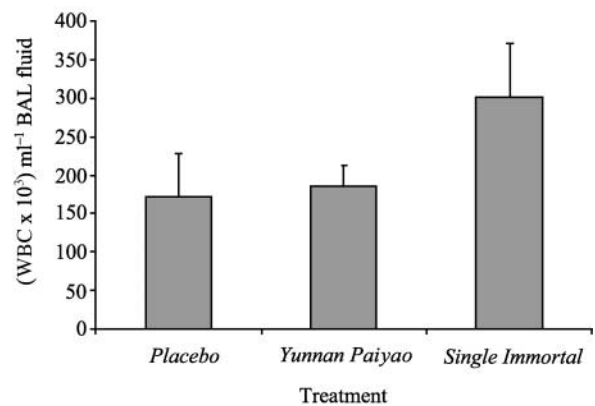


Fig. 2 White blood cells (WBCs) in bronchoalveolar lavage (BAL) fluid following maximal exercise ($n = 5$) after treatment with placebo, *Yunnan Paiyao* and *Single Immortal*. There were no significant differences between conditions ($P > 0.05$)

as a result of treatment with the herbal formulations (Table 2).

Discussion

This is the first study to investigate the impact of herbal formulations on the severity of EIPH. These compounds (i.e. *Yunnan Paiyao* and *Single Immortal*) did not reduce the severity of EIPH in maximally exercising horses. Specifically, the RBC counts in the BAL fluid of horses treated with the herbal formulations did not differ significantly from those of horses treated with a placebo. There was, however, a small but statistically significant increase in the time-to-fatigue after herbal treatment with *Single Immortal* that may suggest the presence of some performance-enhancing properties that are unrelated to the severity of EIPH *per se*.

Rationale for using herbal formulations to treat EIPH

Prolonged blood coagulation during exercise has been cited as a possible factor contributing to EIPH^{21-24,26}. Thus, increased clotting times following exercise-induced injury to the blood-gas barrier could theoretically exacerbate the severity of EIPH as a consequence of delayed sealing of damaged microvessels, thereby allowing an increased volume of blood to be extravasated. Indeed, exercise has been shown to diminish the ability of equine platelets to respond to platelet-aggregating factors (adenosine diphosphate, collagen and platelet-activating factor) in both 'EIPH-positive' and 'EIPH-negative' horses²¹⁻²⁴. Moreover, Bayly *et al.*²⁵ have shown that furosemide reduces the exercise-induced inhibition in platelet-induced aggregation to adenosine diphosphate, which was hypothesized to contribute to furosemide's ability to decrease EIPH.

Table 1 Time-to-fatigue, cardiorespiratory variables, arterial blood gases and acid-base data at maximal exercise. Values are means \pm standard error

Variable	Control	Yunnan Paiyao	Single Immortal
Total time-to-fatigue (s)	670 \pm 10	665 \pm 6	685 \pm 8*
Haematocrit (%)	63 \pm 1	64 \pm 1	63 \pm 1
P _a O ₂ (mmHg)	61.8 \pm 1.6	63.2 \pm 1.5	64.4 \pm 3.1
P _a CO ₂ (mmHg)	61.2 \pm 2.8	62.0 \pm 2.8	64.3 \pm 2.3
pH	7.2 \pm 0.30	7.18 \pm 0.30	7.2 \pm 0.20
Mean peak P _{pa} (mmHg)	93.2 \pm 5.9	94.0 \pm 5.9	92.3 \pm 5.2
VO _{2max} (l min ⁻¹)	75.8 \pm 1.5	76.2 \pm 3.5	74.2 \pm 2.0
VCO _{2max} (l min ⁻¹)	80.7 \pm 4.6	85.3 \pm 3.4	77.9 \pm 5.2
Heart rate (beats min ⁻¹)	212 \pm 4	215 \pm 5	213 \pm 4
Respiratory rate (breaths min ⁻¹)	117 \pm 4	117 \pm 3	117 \pm 3
Plasma lactate (peak; mmol l ⁻¹)	26.6 \pm 2.4	23.3 \pm 2	24.3 \pm 2.4

P_aO₂ – arterial partial pressure of oxygen; P_aCO₂ – arterial partial pressure of carbon dioxide; P_{pa} – pulmonary arterial pressure; VO_{2max} – maximal oxygen uptake at STPD; VCO_{2max} – maximal carbon dioxide production at STPD; STPD – standard temperature and pressure, dry.

*Significantly different from control and Yunnan Paiyao ($P < 0.05$).

Table 2 Coagulation variables. Values are means \pm standard error

Variable	Control	Yunnan Paiyao	Single Immortal
Platelets ($\times 10^3 \mu\text{l}^{-1}$)	146 400 \pm 11 587	146 750 \pm 9035	136 600 \pm 9415
Fibrinogen (mg dl ⁻¹)	220 \pm 20	180 \pm 20	240 \pm 40
Partial thromboplastin time (s)	39.08 \pm 1.99	37.84 \pm 2.17	37.62 \pm 2.12
Partial thromboplastin time minus partial thromboplastin time control (s)	36.06 \pm 0.99	36.56 \pm 1.23	35.28 \pm 1.10
Prothrombin time (s)	9.26 \pm 0.21	9.16 \pm 0.16	9.40 \pm 0.26
Prothrombin time minus prothrombin time control (s)	9.18 \pm 0.16	9.22 \pm 0.25	9.28 \pm 0.21

No significant differences were found in any variable except for a possible trend for shortened bleeding times.

Based on the theory that a haemostatic defect would exacerbate EIPH, herbal formulations that are purported to enhance coagulation have been considered as potential treatments for EIPH. The main herbal ingredient of *Yunnan Paiyao* is *Notoginseng*, which reduces bleeding time²⁹⁻³¹, thrombin time³¹, clotting time^{29,30} and coagulation time³⁸, as well as initiating platelet release^{39,40} and decreasing fibrinogenaemia⁴¹. The main herbal ingredients of *Single Immortal* are *Notoginseng* and *Bletillae*. *Bletillae* is effective as a vascular embolizing agent⁴² (promotion of thrombin formation) as well as in decreasing bleeding time and thrombin time³¹. The rationale from a conventional 'Western' medicine perspective that herbal formulations may be effective for treating EIPH is built on the premise that platelet function may be enhanced, since ingredients in these formulations have been shown to shorten bleeding time²⁹⁻³¹.

Possible explanations for the ineffectiveness of herbal formulations

The inability of herbal formulations to reduce EIPH in the present study may indicate that either impaired haemostasis is not a primary factor in the aetiology of EIPH or that these formulations were not effective in addressing the specific coagulation problem. It should also be realized that diagnoses made by traditional Chinese

medicine differ from 'Western' medicine. Traditional Chinese medicine depends on a holistic system of relationships between externally observed symptoms and internal organs to ultimately determine a pattern of illness *versus* addressing individual symptoms and diseases separately (i.e. EIPH). Therefore, a veterinarian utilizing traditional Chinese methods may assign different diagnoses to each individual horse in this population, and choose slightly different formulations to suit the individual evaluation of each animal. However, veterinarians trained in conventional 'Western' medicine would diagnose all horses as having EIPH and treat them all the same (as was done in the current study) with one of the herbal formulations empirically indicated for the reduction of pulmonary haemorrhage, without taking into consideration the individual patterns of illness expressed by each horse⁴³.

Again, the contention that coagulation is compromised in horses with EIPH should be considered cautiously since horses in the coagulation studies²¹⁻²⁵ were designated as 'bleeders' based upon a history of epistaxis with or without endoscopic evaluation. It has been shown^{1,2}, using BAL, that all strenuously exercised horses bleed to some degree. In accordance with this, statistical analysis of most data in the current literature has shown no difference in coagulation profiles between 'bleeders' and 'non-bleeders'.

With regard to demonstrating conclusively that impaired coagulability exists in exercising horses, major confounding variables include the timing of blood sample collection⁴⁴ (before, during or after exercise, since alterations in coagulation are evident only transiently during exercise), the alteration of coagulation parameters as a result of increased fitness and exercise intensity (which tends to augment fibrinolysis⁴⁴ and decrease platelet function²³) and the fact that *in vitro* processing of samples can artificially alter measurements of coagulation variables themselves⁴⁵ (partial thromboplastin time, prothrombin time and platelet function).

Furthermore, in direct contrast to previous studies (that demonstrated a more pronounced decrease in the response of equine platelets from horses during exercise and those known to be 'bleeders'^{21,24} to aggregating agents such as adenosine diphosphate^{22-24,26}, collagen²⁶ and platelet-activating factor²⁶), Kociba *et al.*⁴⁶ found no association between exercise status and decreased platelet aggregation by adenosine diphosphate, or any alteration in coagulation variables including prothrombin time, partial thromboplastin time and bleeding time. Instead, increases in platelet and fibrinogen concentrations and increased platelet retention with maximal exercise were observed in all horses. Moreover, there was no difference between furosemide and placebo with respect to augmenting any of the haemostatic values (prothrombin time, partial thromboplastin time, bleeding time, platelet and fibrinogen concentration, and platelet retention). In addition, recent data published by Kingston *et al.*^{27,45} demonstrated enhanced platelet aggregation in response to supramaximal exercise. Discrepancies between the results of the coagulation studies in exercising horses may be partially explained by the noteworthy finding in the studies of Kingston *et al.*^{27,28} that sodium citrate is not the anticoagulant of choice for evaluating the effects of exercise on equine platelet function because it clearly inhibits platelet aggregation. Therefore, it would be reasonable to consider the possibility that studies using sodium citrate as the anticoagulant may have falsely implicated diminished platelet function in exercising horses in the aetiology of EIPH. This may also explain why herbal formulations designed to address this proposed coagulation problem failed to mitigate the haemorrhage.

Although no alterations have been demonstrated in prothrombin and partial thromboplastin times following exercise with either 'bleeders' or 'non-bleeders'^{22-24,26,44}, shortened whole-blood clotting times and a trend for shortened prothrombin time and thrombin time^{23,44} have been shown. This is also in agreement with the data of Kingston *et al.*^{27,45}, which suggest that horse blood becomes transiently hypercoagulable

during exercise. However, an increased fibrinolysis that is transient with exercise⁴⁴ has been observed and may attenuate or counterbalance the effects of this hypercoagulability.

The data collected from resting horses in our study demonstrated that coagulability variables including platelet number, prothrombin time and partial thromboplastin time were not altered with the herbal formulations, so they would not be expected to be further altered with exercise. Inferences concerning the effects of herbal treatments on cutaneous template bleeding time (which is dependent on the number and functional ability of circulating platelets responding to vascular injury)⁴⁷ would be speculative, since only a limited number of horses were evaluated using the standardized technique of Kopp *et al.*⁴⁸ (unpublished data) three times before treatment ($n = 6$; bleeding time 483 ± 31 s) and after administration of *Single Immortal* ($n = 2$; bleeding time 413.75 ± 21.75 s; two observations), *Yunnan Paiyao* ($n = 1$; bleeding time 585.0 ± 0.0 s; one observation) and cornstarch ($n = 3$; bleeding time 615.0 ± 74.41 s; three observations). Bleeding times were performed on only a few horses since the primary objective of this study was to determine the efficacy of specific herbal formulations in reducing EIPH as evidenced by RBC counts in BAL fluid. In addition, our data along with that from several studies using this technique have reported high variability and large standard errors between and within animals⁴⁸ that make treatment effects, if they exist, hard to detect⁴⁷. However, from a retrospective point of view, conclusive evidence for shortened bleeding times is of little importance mechanistically since the herbal formulations did not diminish the severity of EIPH.

The overwhelming evidence from the current scientific literature suggests that a primary haemostatic deficiency is not present in the exercising horse^{27,28,45}. This is also supported by the data from the current study, especially since herbal formulations designed to shorten coagulation did not reduce EIPH. Rather, the potential exists for an exercise-induced hypercoagulability with the formation of platelet-neutrophil aggregates^{45,49,50} (evaluated by spontaneous echocardiographic contrast)⁵¹ that may act to increase pulmonary arterial pressure by lodging in the microvasculature and consequently increasing EIPH. In fact, the tendency for EIPH to be increased with herbal treatments in the current study (Fig. 1) suggests that ruptures in the blood-gas barrier are not sealed more effectively with the current treatment.

Further evidence to suggest that a haemostatic deficiency does not exist in exercising horses comes from the work of Elliot *et al.*⁵², in which rapid reversibility of capillary breaks (within 3 min of decreasing capillary pressure, which is less than the clotting time for

horses) was observed and inferences made from the morphological data suggested this to be the result of a mechanical phenomenon (release of tension) rather than biological repair by cells (i.e. platelets). Subsequent data from West *et al.*¹² supported this work, since breaks in the blood-gas barrier of post-maximally exercised horses were difficult to find and were frequently associated with platelets and leucocytes plugging the breaks (implying no problem with coagulation). It has been shown that once the threshold pressure required to cause stress failure of the capillaries^{53,54} is reached, the incidence and severity of capillary breaks do not change as a function of time, suggesting that the degree of haemorrhage is determined simply by the amount of time the 'critical pressure' is exceeded^{15,52} and not by a delay in platelet plug formation.

Possible explanation for increased time-to-fatigue by *Single Immortal*

Time-to-fatigue was increased significantly when *Single Immortal* was administered to the horses versus *Yunnan Paiyao* or the placebo. It therefore appears that *Single Immortal* may have some ergogenic properties that are unrelated to the severity of EIPH. In a recent study, the treatment of horses with *Echinacea*⁵⁵ was found to have potential ergogenic effects as a haematinic agent, due to the increased size and concentration of RBCs in the peripheral circulation and the associated increased concentration of haemoglobin that may enhance oxygen transport and thus performance. However, similar effects were not observed in this study. Huang⁵⁶ reported that endurance was prolonged in animals treated with *Ginseng* root, and it may be that *Single Immortal* contains a larger proportion of the plant (whole herb versus the active ingredient affecting haemostasis) than *Yunnan Paiyao*. It is also possible that an ingredient in *Single Immortal* may have effects that are currently undetermined.

Conclusions

In conclusion, the herbal formulations *Yunnan Paiyao* and *Single Immortal*, when used at the dosage and for the duration evaluated herein, were not effective in reducing EIPH severity in maximally exercising horses. The results of our experiment can be interpreted in at least two different ways. Specifically:

- haemostatic impairment (i.e. platelet function defect) does not contribute significantly to EIPH severity, or alternatively
- the herbal treatments did not successfully address the coagulability anomaly that is actually present.

The potential performance-enhancing capability of *Single Immortal* is worthy of further study to determine:

- if a longer time-to-fatigue may have offset any EIPH benefit derived from herbal treatment and
- the mechanism for this phenomenon.

The findings of the present investigation should not be interpreted beyond the immediate context of the results obtained after treatment with *Yunnan Paiyao* and *Single Immortal* at this particular dose and duration. There also remains the possibility that other herbal formulations may be successful in reducing EIPH.

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