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Deer blood effectively improved clinical signs of anaemia in a rodent model

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Abstract. Iron-deficiency anaemia (IDA) is one of the most common health problems in the world. As a type of traditional Chinese medicine, deer blood (DB) is often used to treat IDA in China. However, no scientifically designed studies with strict controls were available for the evaluation of therapeutic effects of DB on IDA. In the present study, IDA rat model was first established through feeding iron-deficient diet and then three doses of DB treatment (low, mid and high) were used to feed these rats. During the 30-day treatment period, body condition of the negative-control rats continued to decline. There was no significant difference among the groups of DB-high, positive control and overall intact control in haemoglobin concentration, haematocrit concentration and the number of red blood cells. Results showed that both groups DB-mid and DB-high showed significantly increased iron concentrations in the three organs including liver, spleen and kidney of the rats, compared with all other groups, including the positive-control group. We believe our study has opened a new avenue for the development of DB as a drug to treat IDA in clinics.

Additional keywords: iron, iron-deficiency anaemia, model.

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Introduction

Iron-deficiency anaemia (IDA) is considered as one of the most common health problems (Mei *et al*. 2017), particularly for the preschool children and pregnant women. Pregnant women suffering from anaemia have an increased risk of poor birth outcomes, pre-term birth, low birthweight and high neonatal mortality (Rahman *et al*. 2016), and maternal mortality (Wirth *et al*. 2017). Furthermore, growth rate and health condition of children suffering IDA are seriously affected (Stevens *et al*. 2013). IDA also leads to reduced work productivity and poor living conditions (Yan *et al*. 2011).

Iron salts have been used as a therapeutic means to improve IDA symptoms for the past 300 years (Jimenez *et al*. 2015) in western countries. Although this is an effective and fast treatment, adverse effects are frequently encountered, such as gastrointestinal side effects (Mandal and Mukherjee 2017). As a type of traditional Chinese medicine, deer blood (DB) is often used to effectively alleviate or treat IDA in China (Zhihao Z.*et al*. 2013). DB is mainly derived from two deer species, namely, sika deer (*Cervus nippon*) and red deer (*Cervus elaphus*), and is rich in proteins, amino acids (Lei *et al*. 2006), vitamins such as A, B1, B2 and K (Zhihao *et al*. 2013), fatty acids and phospholipids (Shouben *et al*. 1999), steroids such as progesterone, cortisol and estradiol (Changsheng *et al*.2001), androgen (Yuan *et al*.2014), bioamines (Wanchao *et al*. 1998) and inorganic elements (Song and Ge 1999; Baroni *et al*. 2000; Xubin *et al*. 2001). Thus far, no scientifically designed studies with strict controls are available for the exploitation of the therapeutic effects of DB on IDA. In the present study, we investigated the efficacy of DB in improving nutritional anaemia with proper controls, and determined whether overdose of DB could be toxic to rats.

Materials and methods

The study was approved by the Temporary Animal Ethics Committee of Institute of Special Wild Economic Animals and Plants, Chinese Academy of Agricultural Sciences (Permit number: 2018-0013).

Group allocation

In total, 80 8-week-old female SD rats (bodyweight: 200 ± 20 g, purchased from Liaoning Changsheng Biotechnology Co., Shenyang City, China) were randomly allocated to eight groups (10 rats/group). Six groups (model rat group) were fed with a formulated iron-deficient diet (Table 1) to establish IDA rat model. After the establishment of the IDA rat model, five

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groups from IDA model were kept further and fed with an iron-deficient diet for 30 days. Of these five groups, three were treated with DB at the following three different doses: 360 (Group 1), 720 (Group 2) and 1440 mg/kg.day (Group 3); and the remaining two groups were treated with lactate iron at 8.42 mg/kg.day (Group 4) and sodium carboxymethyl cellulose solution (CMC-Na, Group 5). The left-over group of non-model rats was switched to a standard diet from iron-deficient diet (Group 6). Lactate iron and DB were all dissolved in 1 mL 0.5% CMC-Na before gavage. One extra control group (Group 7) was set aside and fed with standard diet, the other extra control group (Group 8) was treated with an overdose of DB (1440 mg/kg.day) to determine whether DB was toxic to rats. The group allocation is listed in Table 2.

DB collection

Deer blood was collected from two 4-year-old male sika deer during the period of velvet antler growth, under general anaesthesia (xylazine hydrochloride, 1.5–2.0 mL/100 kg bodyweight), through jugular vein by using vacuum blood-collection device. Thereafter, anaesthesia was reversed using Nikethamide (1.5–2.0 mL/100 kg bodyweight). DB was dried at 65°C for 24 h in a drying oven immediately after collection, and then ground into ultrafine powder (300 mesh).

Table 1. Composition of the iron-deficient diet

Item	Percentage
Glucose	49.4
Albumin	20.0
Corn oil	5.0
Corn flour	15.0
Sodium dihydrogen phosphate	2.0
Calcium carbonate	2.0
Potassium chloride	0.5
Trace-element mix[A]	10.3
DL-methionine	0.3
Gelatin	5.0
Vitamin mix[B]	20.1
Choline chloride	0.2

[A]$MgSO_4 \cdot H_2O$, 73.816 g; $ZnSO_4 \cdot 7H_2O$, 19.657 g; $MnSO_4 \cdot H_2O$, 5.733 g; $CuSO_4 \cdot 5H_2O$, 0.7315 g; KIO_3, 0.0625 g.

[B]Vitamin A (500k IU/g), 1.00 g; vitamin D3 (200k IU/g), 0.75 g; α-tocopheryl acetate (25% vitamin E in albumin), 12.50 g; vitamin K, 0.04 g; thiamine hydrochloride, 0.30 g; riboflavin, 0.30 g; vitamin B6, 0.30 g; generic dairy calcium, 0.60 g; niacin, 3.00 g; folate, 0.10 g; vitamin B12 (0.1% vitamin B12 in albumin), 2.00 g; sucrose 79.11 g.

Establishment of rat IDA model

To establish the IDA model, rats were fed with a formulated iron-deficient diet (3.7 mg Fe/kg, Table 1), to speed up the course of model establishment, bloodletting was performed from caudal vein every 5 days. Whether the establishment of the model was successful was judged from the colour of eyes, ears and claws, in combination with haemoglobin (HGB) concentration in tail vein blood (Gao *et al.* 2019). Sixty days after the commencement of the experiment, HGB concentration had dropped below the threshold, i.e. 100 g/L blood, and the model establishment was considered to be successful (Yan *et al.* 2009). The two extra control groups (Groups 7 and 8) were fed with standard rat diet (149 mg Fe/kg).

Oral medication was administered after the establishment of the IDA rat model, and the rats were weighed, and the concentrations of HGB and haematocrit (HCT), and the number of red blood cells (RBC) of the rats were determined every 10 days. The doses for the three treatment groups (Groups 1–3) were equivalent to 5, 10 and 20 times of those for adult humans respectively.

Measurement of the concentrations of HGB and HCT, and determination of the RBC count

Rats were weighed before each blood collection from the tail vein every 10 days, and the HGB concentration, HCT concentration and the number of RBC in the rat blood were determined. At the end of the experiment and before sacrificing, rats were fasted for 12 h, and blood was then collected directly from rat hearts after anaesthesia (0.7 mL 10% chloral hydrate), and centrifuged to obtain serum. Measurement of the concentrations of HGB and HCT, and determination of the RBC count were performed using automatic blood analyser (URIT-2900Vet Plus, URIT Medical Electronic Co., Nanning, China).

Histological examination

Liver, spleen, heart and kidney were collected immediately after the rats were sacrificed, and each of those organs was divided into two parts. One part was fixed in 10% buffered

Table 2. Animal-group allocation and description

CMC-Na, sodium carboxymethyl cellulose solution. Note that DB and lactate iron were all dissolved in 1 mL 0.5% CMC

Type	Group	Diet description	Diet item	Dose (mg/kg.day, unless otherwise stated)
Model	1	DB-low	Deer blood	360
	2	DB-mid	Deer blood	720
	3	DB-high	Deer blood	1440
	4	Positive control–lactate iron	Lactate iron	8.42
	5	Negative control–CMC-Na	0.5% CMC-Na	1 mL/rat.day
	6	Model-control–standard diet	Iron (Fe)	149 mg/kg diet
Extra control	7	Intact–standard diet	Fe	149 mg/kg diet
	8	DB-high	Deer blood	1440

formalin immediately for histological examination. The tissue samples were embedded in paraffin wax, sectioned at 5 μm, stained with haematoxylin and eosin, and examined under a microscope (IX83, OLYMPUS, Japan). Another part was rinsed with phosphate buffer solution (pH = 6.86) and used for the measurement of iron concentration.

Measurement of iron concentration

Different diet and organ samples were dried in an oven at 100°C for 24 h until a constant weight was reached, after which they were weighed (approx. 0.5 g of each) and digested for iron concentration measurement according to the methods described in GB/T 13885-2017, by using AAS instrument (ZEEnit700, Analytikjena, Jena, Germany).

Statistical analyses

The data are presented as means ± s.d. Statistical analysis was performed using Student's *t*-test. Significance was set at $P < 0.05$.

Results and discussion

Iron concentration in DB powders and diets

Our analysis results showed that iron concentration in the iron-deficient diet (3.73 ± 0.19 mg Fe/kg) was significantly ($P < 0.05$) lower than that of the standard diet (149 ± 4.55 mg Fe/kg). Iron concentration of DB (1732 ± 54.41 mg Fe/kg) was significantly ($P < 0.0001$) higher than that of the standard diet (149 ± 4.55 mg Fe/kg).

Evaluation of the rat IDA model

Bodyweight of rats did not differ significantly ($P > 0.05$) among the groups at the time of the commencement of the experiment. Rat HGB and HCT concentrations and rat RBC count of each of the model groups after the model establishment were significantly ($P < 0.01$) lower than those at the time of the commencement of the experiment, whereas bodyweight was significantly increased (from 213.7 g to 266 g), although the growth rate of the model groups (0.87 g/day) was significantly slower than that (1.59 g/day) of the two extra control groups (Groups 7 and 8; Table 3). In contrast, there were no significant ($P > 0,05$) changes in HGB, RBC and HCT in the rats of the two extra control groups between the time before and after the model establishment (timing was based on the model group).

Sixty days after feeding the iron-deficient diet, bodyweight and HGB concentration in the IDA model rats were significantly ($P < 0.001$) lower than those in the two extra control groups (Groups 7 and 8; Figs 1, 2). Consequently, typical symptoms of IDA were achieved in the rats in the model groups.

Bodyweight gain and change in the concentrations of HGB and HCT, and in the RBC count

Results of the routine blood tests are shown in Fig. 3. Bodyweight (Fig. 3*a*) and HGB concentration (Fig. 3*b*) in Group 6 increased significantly at the end of the experiment, suggesting that the way we established the IDA model did not cause irreparable damage. Rat bodyweight gain occurred in all groups through eating supplemented DB, especially in Group

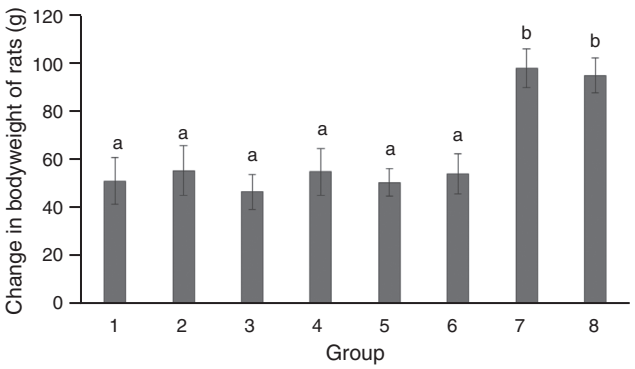

Fig. 1. Changes in rat bodyweight before and after the model establishment. Bars with different letters are significantly different (at $P = 0.05$). Groups 1–3, deer blood (DB)-low, DB-mid, and DB-high respectively; Group 4, positive control–lactate iron; Group 5, negative control–CMC-Na; Group 6, model control–standard diet; Group 7, control intact–standard diet; Group 8, control DB-high.

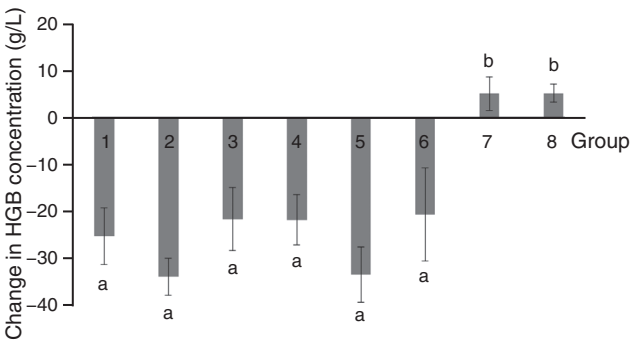

Fig. 2. Changes in haemoglobin (HGB) concentration in the iron-deficient anaemic rats before and after the model establishment. Bars with different letters are significantly different (at $P = 0.05$). For definition of Groups 1–8, refer to Fig. 1 or Table 1.

Table 3. Bodyweight, haemoglobin (HGB) and haematocrit (HCT) concentration, and red blood cell (RBC) count before and after model establishment ($\bar{x} = n \pm n$)

Values within a column followed by the same letter are not significantly different (at $P = 0.05$)

Group	Day	Bodyweight (g)	HGB (g/L)	RBC ($\times 10^{12}$/L)	HCT (%)
Model	0	213.7 ± 8.58a	130 ± 4.53a	7.00 ± 0.25a	36.37 ± 1.45a
	60	266 ± 15.86a	95.9 ± 8.7b	6.30 ± 0.52b	23.75 ± 2.87b
Extra control	0	213.4 ± 8.41a	130.7 ± 4.11a	7.03 ± 0.26a	36.63 ± 1.44a
	60	308.9 ± 17.96a	135.3 ± 2.83a	7.11 ± 0.22a	34.74 ± 0.93a

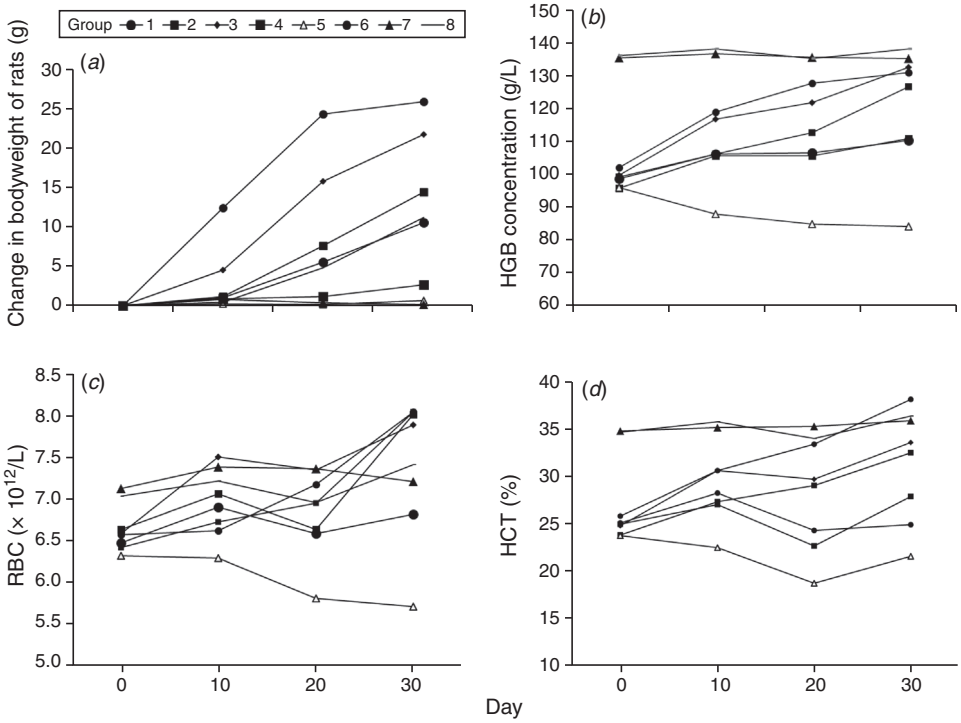

Fig. 3. Change in bodyweight, haemoglobin (HGB) and haematocrit (HCT) concentrations and red blood cell (RBC) count of the rats at 0, 10, 20 and 30 days from the start of feeding deer blood (DB). For definition of Groups 1–8, refer to Fig. 1 or Table 1.

3. There was no significant difference in bodyweight between Group 3 and Group 6 30 days after the treatment. As an extra control group, bodyweight gain in Group 8 significantly increased, while that in Group 7 did not (Fig. 3).

As shown in Fig. 3, HGB concentration, RBC count (Fig. 3*c*) and HCT concentration (Fig. 3*d*) in the IDA model rats increased with an increasing DB dosage, that is, dose-dependent results during the 30-day DB treatment were achieved. The HGB concentration of Group 3 increased the most compared with the other groups ($P < 0.01$). The HGB concentration of Group 5 decreased (from 95.88 to 84.13 g/L), while that in the other groups increased (all above 110 g/L), indicating that a diet without a sufficient iron concentration would lead to an emaciated physique, lowered bodyweight and a lowered HGB concentration. Ten days after the DB treatment, the RBC count and the concentrations of HGB and HCT in Groups 1–4 increased significantly ($P < 0.05$). Thirty days after the DB treatment, no significant ($P > 0.05$) difference in the RBC count, or the concentrations of HGB and HCT was found among Groups 3, 4 and 7, indicating that the anaemia caused by iron deficiency was significantly improved. It has been reported that in the iron-deficient regions, such as in some Mediterranean countries (Cordero *et al*. 2019), children normally have lower bodyweights, lower concentrations of HGB and HCT, and a lower RBC count (Olivieri and Brittenham 1997). These symptoms resemble those occurred in our IDA rats. Therefore, we believe that DB can be used as an alternative diet supplement to effectively alleviate symptoms of anaemia in these children.

Change in the iron concentration of liver, spleen and kidney

It has been reported that the main storage organs for iron are liver, spleen and kidney (Solecki *et al*. 1983); iron in these organs is metabolised and converted into a functional form, HGB molecules, for body to use. As shown in Fig. 4, liver iron did not significantly ($P > 0.05$) increase in Group 4 rats 30 days after the treatment. However, in the three DB-treated groups, the concentration of the liver iron increased to a level similar to that of Group 7 (standard diet). Likewise, change in the iron concentration of the kidney had a similar trend. A significant increase in the iron concentration of the kidney was observed in Groups 2 and 3 during the 30-day treatment period, while a similar trend was not observed in Groups 1, 5 and 8 ($P > 0.05$). There was no significant ($P > 0.05$) difference in the iron concentration of the spleen between the three DB-treated groups and Group 7, while that of Group 7 was significantly higher than that of Group 8 ($P < 0.05$). Iron concentrations in the liver, spleen and kidney of the DB-treated groups were significantly ($P < 0.05$) higher than those of Groups 4 and 5. An increased need for iron in the body causes a rapid catalysis of ferritin in liver, spleen and kidney (Robscheitrobbins *et al*. 1935). This is probably why concentrations of iron in the liver, spleen and kidney of the IDA rats were significantly lower than those in the rats fed with standard diet in the present study. Therefore, DB supplementation would provide certain amount of iron to these organs of the IDA rats, for the leftover iron that would be converted to HGB (Huh *et al*. 1999).

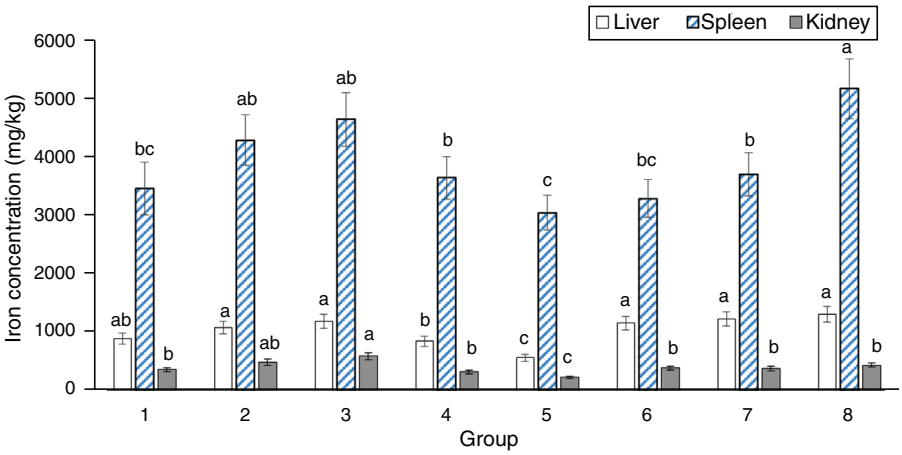

Fig. 4. Iron concentration in liver, spleen and kidney of the rats. For definition of Groups 1–8, refer to Fig. 1 or Table 1. Bars with different letters were significantly different (at $P = 0.05$).

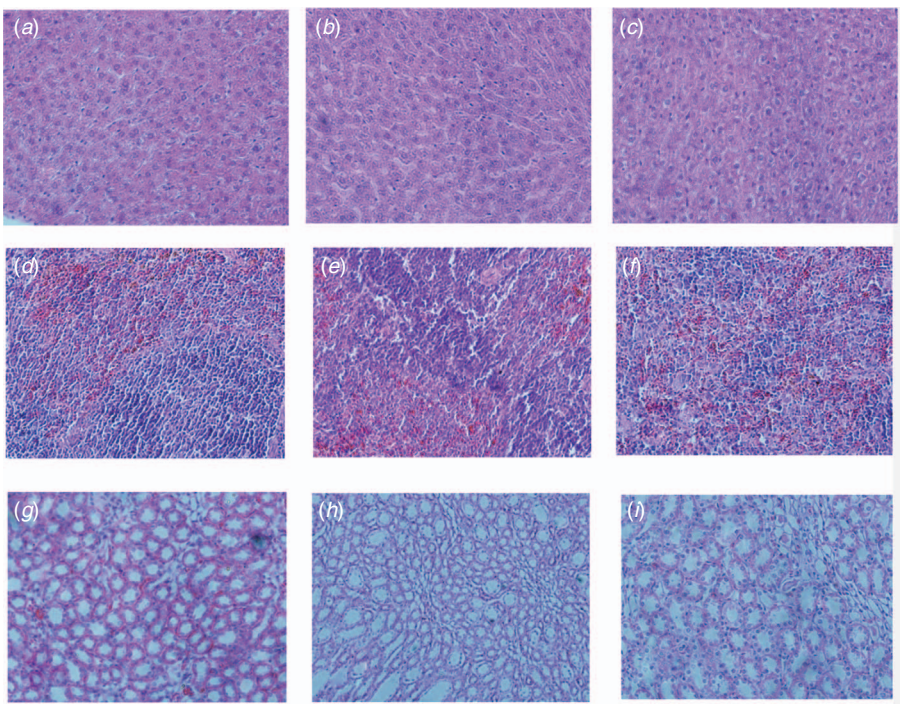

Fig. 5. Histologic sections of liver, spleen and kidney in the extra control group (Group 7) that was set aside and fed with standard diet, in the other extra control group (Group 8) that was treated with an overdose of deer blood, and in the iron-deficiency anaemia (IDA) model group that was treated with an overdose of deer blood (Group 3). (*a–c*) Liver, ×20; (*d–f*) spleen, ×40; (*g–i*) kidney, ×40.

Changes in the histological structure of liver, spleen and kidney

To verify whether the overdose of the DB treatment has side effects to some organs in rats, histological examination of liver, spleen and kidney from Groups 7, 8 and 3 was undertaken. Results (Fig. 5) showed no observable difference in the histological structure among Groups 3, 8 and 7, suggesting that high doses of DB did not cause detectable damage to rats.

Conclusions

Using our successfully established IDA model, we found that DB treatments effectively increased iron concentrations in the liver, spleen and kidney in rats. Bodyweight, HGB and HCT concentrations, and the RBC count in the DB-high group were significantly higher than those of the DB-mid, DB-low and positive-control groups; therefore, the DB-high is considered as the optimal dose for the treatment of IDA in the future.

Conflicts of interest

The authors declare no conflicts of interest.

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