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Study on the Therapeutic Effect of Modified Yujin Powder on *E. coli* in the *Procapra przewalskii*

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ABSTRACT

This experiment was to study the therapeutic effect of modified Yujin powder on *E. coli* in the *P. przewalskii*. Twenty the *Procapra przewalskii* with the *E. coli* were selected from the Wildlife Rescue Center of the Qinghai Lake National Nature Reserve and randomly divided into low-dose group, high-dose group, gentamicin sulfate group and control group with 5 *Procapra przewalskii* in each group. The *P. przewalskii* in modified Yujin powder group were given Yujing powder once every morning and evening, 3, 4 mL/ (kg·BW) for the low-dose group and high-dose group, respectively. The *P. przewalskii* were injected with gentamicin sulfate intramuscularly,once every morning and evening. The *P. przewalskii* in control group were fed normally and no medicine was given. After 7 d, the clinical symptoms of the *P. przewalskii* were observed, and the blood routine, serum biochemical parameter, serum immune and antioxidant indices were measured. The results showed that the *P. przewalskii* were sensitive reaction, increased appetite, and no diarrhea in the modified Yujin powder group. The healing rates were 75%, 85%, and 60% in low-dose group, high-dose group, gentamicin sulfate group, respectively. The effects of modified Yujin powder was better than gentamicin sulfate. The contents of blood urea nitrogen (BUN), malondialdehyde (MDA), and the number of white blood cell (WBC) in 3 drug delivery groups were extremely significantly lower ($P<0.01$) than the control group, and the contents of potassium (K), total protein (TP), albumin (ALB), globulin (GLB), and total antioxidant capacity (T-AOC) in the 3 drug delivery groups were extremely significantly higher ($P<0.01$) than that of control group, but there were no remarkable differences between the 3 drug delivery groups. The serum catalase (CAT) activity in the high-dose group was extremely significantly higher ($P<0.01$) than that in the low-dose group, gentamicin sulfate group, and control group. Consequently, modified Yujin powder had a good therapeutic effect on the *E. coli* in *P. przewalskii*, and the effect of the high-dose group was better.

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CJS collected material, compiled the results and writing of manuscript. CJS and SQG performed the experiments. XYS reviewed the manuscript.

Key words
The *E. coli*, The *P. przewalskii*, Modified Yujin powder, Gentamicin sulfate, Blood parameter

INTRODUCTION

The Przewalski's gazelle (*Procapra przewalskii*) is endemic to the Qinghai-Tibetan Plateau, China, and has been widely distributed throughout the Inner Mongolia, Ningxia, Gansu, Qinghai, Xinjiang, and Tibet regions (Hu and Jiang, 2011; Ping *et al*., 2018). In recent years, the distribution range of the *P. przewalskii* has been gradually shrinking because of human population growth, grassland degradation, and habitat fragmentation (Shen *et al*., 2018; Chi *et al*., 2020; Jiang, 2004). Currently, only the Qinghai Lake basin remains preserved as a habitat for the *P. przewalskii* (Yang *et al*., 2006). According to the survey results in recent years, only the *P. przewalskii* populations comprising about 300 animals inhabit the Qinghai Lake area (Zhang *et al*., 2018). Therefore, the *P. przewalskii* has become the least populous species among the endemic mammals in China (Shen *et al*., 2009, 2019; Jiang and Wang, 2000. In 2008, the *P. przewalskii* was recategorized on the International Union for Conservation of Nature (IUCN) Red List from "critically endangered" to "endangered" (Li *et al*., 2012).

The colibacillary *(Escherichia coli)* is an acute bacterial infection caused by infection with pathogenic *E. coli* (Fernando *et al*., 2019; Wu, 2014). It is characterized by depression, loss of appetite, abnormal growth and development, diarrhea, and high mortality (Fit *et al*., 2019; Mainil, 2013). The main characteristics of affected animals are to excrete the feces with foul smell and gray color (Ma, 2017). The disease will not only

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cause dehydration and debilitation, but also increase the mortality of *P. przewalskii*. At present, the treatment of *E. coli* is mainly based on antibiotics, but there are problems such as drug residues and drug resistance (Su *et al.*, 2019). In recent years, the research and development of Chinese herbal medicine antibacterial and antiviral drugs has become a research hot spot at domestic and abroad (Tong *et al.*, 2014). Traditional Chinese medicine can not only directly kill bacteria, but also exert antibacterial effect by enhancing the organism immunity. Traditional Chinese medicine has the dual functions of medicinal and nutrient in clinical practice, which is environmentally friendly, safe and resistant to drug resistance (Ning *et al.*, 2015; Ho *et al.*, 2013). Chinese herbal medicines have small side effects, low prices, and wide sources, which can also reduce the cost of breeding (Tong *et al.*, 2014; Meng *et al.*, 2015).

According to the Chinese veterinary syndrome theory, *E. coli* is caused by damp-heat hoarding of the intestinal tract, which should be heat-clearingand detoxifying, eliminating dampness and relieving diarrhea (Li, 2017). Yujin powder is the prescription in "Yuan Heng Liao Ma Ji" (Commission of Chinese veterinary pharmacopoeia, 2011). It has the effects of heat-clearingand detoxifying, dispersing phlegm and stopping diarrhea (Liao *et al.*, 1990). This experiment adds 4 Chinese herbal medicines of *Atractylodes macrocephala*, *Glycyrrhiza uralensis*, *Common vladimiria* and *Pulsatilla chinensis* on the basis of Yujin powder, and subtracts *Scutellaria baicalensis*, *Phellodendron amurense*, *Gardenia jasminoides* and *Rheum palmatum*. Take Yujin to promote and normalize the flow of qi as a principle drug. *Atractylodes macrocephala*, *Glycyrrhiza uralensis*, and *Common vladimiria* can regulating qi for invigorating spleen, as an adjuvant drug. *Coptis chinensis* and *Pulsatilla chinensis* can clear heat, eliminating dampness, and removing toxicity, as an assistant drug. *Terminalia chebula* relieving diarrhea with astringents. *Cynanchum otophyllum* retaining yin with astringent and harmonizing method (Li, 2002). The combination of various drugs has the effects of clearing away heat and detoxifying, relieving diarrhea with astringents. By studying the therapy of modified Yujin powder on *E. coli* in *P. przewalskii*, it aims to provide a reference for clinical treatment of such bacterium, and also provides new ideas for the protection of the *P. przewalskii*.

MATERIALS AND METHODS

Preparation of traditional Chinese medicine and gentamicin sulfate

Modified Yujin powder is composed of tulip, *S. baicalensis* and *G. uralensis*, each 25 g. *A. macrocephala*, *C. vladimiria*, *P. chinensis*, and *C. otophyllum*, each 20 g, and *T. chebula* 15 g. Soak for 30 min with water, boil quickly with a fire, then use a simmer to cook. The drug juice was filtered, and the filtered drug residue and the appropriate amount of water were further boiled for 30 min, and then filtered, and the filtrate was combined and concentrated to contain 1 g of crude drug per 1 mL of the drug solution.

Experimental animals

The experimental animals were selected from the Wildlife Rescue Center of the Qinghai Lake National Nature Reserve. *E. coli* antibody test was carried out on *P. przewalskii*, and twenty animals (1.5-year-old) with negative antibody detection and clinical symptoms were selected. Nutrition level of grassland types in the habitat of *P. przewalskii* as shown in Table I.

Table I. Nutrition level in main grassland types of *P. przewalskii* (%).

Category	CP	EE	CF	NFE
Artemisia desertorum shrubland	11.36	3.86	30.88	33.94
Agropyron cristatum grassland	9.16	2.36	35.63	45.99
Iris lacteal grassland	12.10	2.81	29.00	33.70
Achnatherum splendens grassland	10.96	2.94	26.06	49.18
Potentilla discolor grassland	14.89	3.06	18.17	47.53
Stipa purpursa grassland	13.10	3.00	27.89	42.52
Elymus nutans grassland	9.90	2.73	28.37	48.65
Carex rigescens grassland	11.44	2.80	28.35	45.27
Orinus kokonorica grassland	14.98	4.07	21.05	41.17
Stipa breviflora grassland	13.80	3.61	28.28	38.36

CP, crude protein; EE, ether extract; CF, crude fiber; NFE, nitrogen free extract.

Experimental design

Twenty *P. przewalskii* with *E. coli* were randomly divided into 4 groups. Modified Yujin powder were low-dose group and high-dose group. According to the low (3 mL/kg, BW), high (4 mL/kg, BW) dose of intragastric administration, each morning and evening, dosing for 7 d. The gentamicin sulfate group intramuscular injection of 10 mL gentamicin sulfate 0.1-0.2 mL/kg, twice a day, for 7 d. The control group was fed normally and was not administered. The test period was 14 days, and the drug was administered for 7 d, observe for 7 d.

Judgment of drug effect: After healing, the clinical symptoms of the affected animals completely disappeared, the affected animals were react quickly, with a strong appetite, no diarrhea, and no recurrence within 7 d after stopping the drug. Effective is that the main clinical symptoms of the affected animals, has a good mental state, increased appetite, and reduced diarrhea, but relapsed within 7 d after stopping the drug. Invalidation is that the clinical symptoms of the affected animals were not alleviated after treatment, and there was a tendency to increase.

Sample collection

At the end of the experiment, blood samples from 20 affected animals were collected from the jugular, placed in a heparin sodium vacuum tube for 60 min, centrifuged at 4 °C, 3500 r/min for 10 min, and stored at -80°C for testing.

Physiological and biochemical indicators

Hemoglobin (Hb), erythrocyte count (RBC), packed cell volume (PCV), corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and white blood cell count (WBC) were determined by automatic blood cell analyzer (SF-3000, Sysmex-Toa Medical Electronics, Kobe, Japan). Biochemical analyses, which included blood urea nitrogen (BUN), aspartate aminotransferase (AST), alanine transaminase (ALT), superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px), malondialdehyde (MDA), total antioxidant capacity (T-AOC), potassium (K), magnesium (Mn), and phosphorus (P) were determined by automatic biochemical analyzer (SF-1, Shanghai Medical Apparatus and Instruments Factory, Shanghai, China). Total protein (TP), albumin (ALB), globulin (GLB) were determined by electrophoresis (WH-300-LCD, Shanghai Shuangqi Biotechnology Co., Ltd., China).

Statistical analyses

Data were analyzed using the Statistical Package for the Social Sciences (SPSS, version 22.0, Inc., Chicago, IL, USA) and presented in the form of mean±standard error (SE). The differences were assessed by Student's *t*-test and were extremely significant at $P<0.01$.

RESULTS

The incubation period is 1-2 d, mostly occurs in 6-week-old *P. przewalskii*. At the beginning, the body temperature of the juvenile *P. przewalskii* increased to above 41°C, conjunctiva became red and congested, and some of the affected juvenile *P. przewalskii* had neurological symptoms, stiffness of juncture, molars and dyskinesia. There are diarrhea symptoms. First, the yellow and white atheroma loose stool is discharged, and then the yellow and white watery loose stool is discharged. The undigested clot, exfoliated intestinal mucosa and blood silk are often mixed in the stool, and there are a lot of bubbles in the stool. Most of them died within 4-12 h after the onset of the disease, with a mortality rate of 80%. Affected *P. przewalskii* have abdominal pain symptoms, cannot stand, because of long-term diarrhea, and hindquarters were fecal pollution. Adult *P. przewalskii* have different symptoms, some of which are not obvious, some of which are mental depression, anorexia, diarrhea and almost no death. The pathological changes were mainly concentrated in gastrointestinal tract, presenting acute enteritis and systemic anemia. In the stomach, there are a lot of milk clots digested by the stomach, and the gastric mucosa is highly congested and edematous. The contents are in the form of blood and water. Some of the gastric mucosa falls off, the mesenteric lymph nodes are highly congested and swollen, the section is juicy, and the color of the kidney and liver is lighter. There are a lot of bleeding points under the renal capsule and the liver capsule, and the gallbladder is full. There is a large amount of hemorrhage in the endocardium. The whole blood is thin and cannot coagulate normally, and the spleen is significantly enlarged.

As shown in Figure 1, the cure rates of the low-dose and high-dose groups were 75% and 85%, respectively, and the cure rate of the gentamicin sulfate group was 60%. The effects of modified Yujin powder was better than gentamicin sulfate, however, the clinical symptoms of the *P. przewalskii* were still obvious.

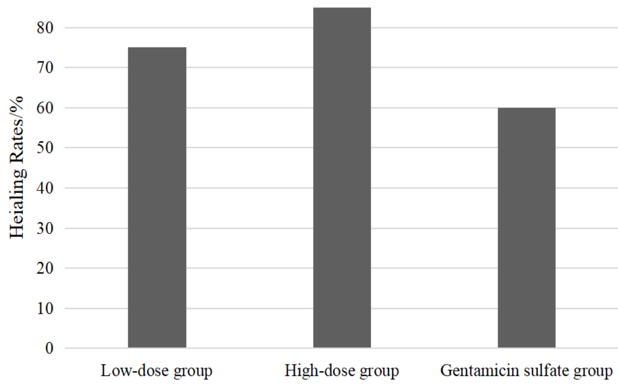

Fig. 1. Analysis of total curative effect.

As shown in Table II, the number of WBC in the low-dose group, the high-dose group, and the gentamicin sulfate group were extremely significantly lower ($P<0.01$) than those in the control group, but the difference between the experimental groups were not significant. There were no significant differences in other blood parameters between groups.

As shown in Table III, BUN and MDA contents in the low-dose group, the high-dose group, and the gentamicin

Table II. Hematological values of *P. przewalskii.*

Items	Low-dose group	High-dose group	Gentamicin sulfate group	Control group
Hb (g/L)	232.56±12.72	231.52±11.33	232.56±12.43	235.63±12.53
WBC (×10^{9}/L)	11.87±0.47^{B}	14.69±0.66^{B}	11.08±0.64^{B}	28.02±3.65^{A}
RBC (×10^{12}/L)	12.88±0.63	12.82±0.55	13.73±0.74	13.76±0.88
PCV (%)	61.86±3.36	62.75±3.36	61.26±3.86	61.83±3.86
MCV (fL)	45.86±0.73	45.88±0.39	45.27±0.74	44.29±0.92
MCH (pg)	16.25±0.35	15.53±0.24	16.43±0.35	15.25±0.54
MCHC (g/L)	344.37±7.69	345.38±7.53	344.57±6.59	343.95±7.85

Hb, hemoglobin; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; MCV, corpuscular volume; PCV, packed cell volume; RBC, erythrocyte count; WBC, white blood cell count. Different capital letter superscripts indicate extremely significant differences at *P*<0.01 level.

Table III. Serum biochemical indices of *P. przewalskii.*

Items	Low-dose group	High-dose group	Gentamicin sulfate group	Control group
BUN (mmol/L)	1.79±0.33^{B}	1.83±0.42^{B}	1.87±0.34^{B}	6.89±0.46^{A}
AST (U/L)	89.87±8.47	89.75±8.32	89.08±8.64	88.52±8.65
ALT (U/L)	44.64±4.74	44.53±4.75	44.58±4.74	43.84±4.75
GSH-Px (U/mL)	114.64±21.35	113.64±21.36	113.64±21.85	112.64±21.86
SOD (U/mL)	94.43±9.24	95.75±9.74	94.73±9.62	94.84±7.63
CAT (U/mL)	16.64±1.43^{B}	18.64±1.86^{A}	16.63±1.24^{B}	14.32±1.54^{C}
MDA (nmol/L)	20.64±2.54^{B}	24.53±2.32^{B}	21.53±2.51^{B}	33.64±2.25^{A}
T-AOC (U/mL)	3.42±0.22A	4.95±0.32^{A}	3.64±0.12^{A}	2.52±0.21^{B}
K (mmol/L)	3.51±0.22^{A}	3.52±0.22^{A}	3.55±0.20^{A}	2.41±0.12^{B}
Mn (mmol/L)	0.30±0.067	0.29±0.015	0.29±0.058	0.29±0.067
P (mmol/L)	2.85±0.43	2.83±0.34	2.84±0.25	2.82±0.54

BUN, blood urea nitrogen; AST, aspartate aminotransferase; ALT, alanine transaminase; GSH-Px, glutathione peroxidase; SOD, superoxide dismutase; CAT, catalase; MDA, malondialdehyde; T-AOC, total antioxidant capacity; K, potassium; Mn, magnesium; P, phosphorus. Different capital letter superscripts indicate extremely significant differences at *P*<0.01 level.

Table IV. Serum immunologic parameters of *P. przewalskii.*

Items	Low-dose group	High-dose group	Gentamicin sulfate group	Control group
TP (g/L)	56.76±5.78^{A}	57.62±5.66^{A}	56.53±4.64^{A}	36.20±3.78^{B}
ALB (g/L)	31.82±2.56^{A}	31.52±3.65^{A}	30.62±3.64^{A}	23.78±3.99^{B}
GLB (g/L)	24.67±2.71A	24.41±2.52^{A}	23.51±2.53^{A}	14.42±2.23^{B}
A/G	1.29±0.16	1.29±0.25	1.30±0.19	1.64±0.23

TP, total protein; ALB, albumin; GLB, globulin; A/G, albumin/globulin. Different capital letter superscripts indicate extremely significant differences at *P*<0.01 level.

sulfate group were extremely significantly lower (*P*<0.01) than those in the control group, while K content and T-AOC level were greatly significantly higher (*P*<0.01) than those in the control group. There was no significant difference between the experimental groups. The activity of CAT in the high-dose group was extremely significantly higher (*P*<0.01) than that in the low-dose group, the gentamicin sulfate group, and the control group. The activity of CAT in the low-dose group and the gentamicin sulfate group was greatly significantly higher (*P*<0.01) than that in the control group. The difference between the low-dose group and the gentamicin sulfate group was not significant. There were no significant differences in other biochemical indicators between groups.

As shown in Table IV, TP, ALB, and GLB contents in the low-dose group, the high-dose group, and the gentamicin sulfate group were extremely significantly higher (*P*<0.01) than those in the control group, but the difference between the experimental groups was not significant.

DISCUSSION

Yang *et al*. (2004) study shows that Yujin has a protective effect on the gastrointestinal tract. Yujin also has the effect of relieving cold diarrhea, and compatibility with *P. chinensis* can reduce the adverse stimulation of the cold of the *P. przewalskii* (Lu *et al*., 2017). Shi *et al*. (2007) study shows that *A. macrocephala* has the effect of regulate qi-flowing for strengthening spleen, eliminating dampness and diuresis, and can improve the organism immune function. Shao *et al*. (2005) showed that *C. vladimiria* has an effect of promoting bile flow. *C. vladimiria* is warm in nature, and it has the effect of relieving pain and regulating the stagnation. It can be used in combination with *A. macrocephala* to treat "qi" deficiency of spleen and stomach, transport weakness, and vomite and eat less. Studies have shown that *T. chebula* and *C. chinensis* have anti-inflammatory effects (Cui *et al*., 2015; Li *et al*., 2015). Therefore, modern pharmacology believes that *C. chinensis* and *T. chebula* have a good inhibitory effect on *E. coli*, they have the functions of clearing heat and removing toxicity, and astringent. *C. otophyllum* can supplement blood, and *G. uralensis* can enhance palatability.

Animal blood physiological and biochemical indicators can not only reflect the physiological and health status of animals, but also can be used as one of the indicators to reflect the organism metabolic status (Chuku and Uwakwe, 2012; Saban, 2019; Allam *et al*., 2020; Fasae *et al*., 2015). The number of WBC in blood of the case greatly significantly increased, which may cause damage to the immune system of the organism. BUN is the final product of protein breakdown, which can more accurately reflect the balance between protein metabolism and amino acids in animals (Huo *et al*., 2020; Akhtar *et al*., 2020; Scott *et al*., 1982). In addition, the organism can maintain the balance of organism fluids through the kidneys and maintain the internal environment stability, and the changes in the organism fluids during abnormal renal function (Wang *et al*., 2014; Meng *et al*., 2019), such as vomiting, diarrhea, gastrointestinal drainage, can cause excessive potassium loss in the gastrointestinal tract. Modified Yujin powder can greatly significantly reduced BUN content and WBC, and extremely significantly increased K content. The levels of TP, ALB and GLB in serum can reflect the immune status of the animal's organism (Ryabchenko *et al*., 2018). The results showed that modified Yujin powder can extremely significantly increased the contents of TP, ALB, and GLB. Therefore, modified Yujin powder can reduce the immune function of the *P. przewalskii*. The activities of GSH-Px, SOD, and CAT in serum reflect the organism antioxidant properties and ability to scavenge free radicals (Sinan *et al*., 2017; Song *et al*., 2020). Serum T-AOC level represents the total antioxidant capacity of the organism (Liu *et al*., 2019a, b). Serum MDA is a product of excessive oxidative free radicals attacking the rich unsaturated fatty acids in biofilms (Mahfuz *et al*., 2020; Iqra *et al*., 2019). It is a sensitive and reliable marker reflecting oxidative damage of tissues and cells caused by reactive oxygen species in organisms (Zeng *et al*., 2020; Allam *et al*., 2019). The study showed that the high-dose group can highly significantly increased the CAT activity, and was extremely significantly higher than the low-dose group and the gentamicin sulfate group. Compared with the control group, the high-dose group can extremely significantly reduced the MDA content, and greatly significantly increased the T-AOC. It indicated that the high-dose group can improve the scavenging ability of free radicals, reduce the damage of free radicals to cells, and effectively improve the antioxidant capacity of the *P. przewalskii*.

CONCLUSION

Modified Yujin powder had a good therapeutic effect on the *E. coli* in *P. przewalskii*, and the effect of the high-dose group was better. Modified Yujin powder can effectively enhance the immune and antioxidant functions of *P. przewalskii*, but the research on the active ingredients between Chinese medicine has yet to be further developed.

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Statement of conflict of interest

The authors have declared no conflict of interest.

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