Quantification of Essential Nutrients and Heavy Metals in Modified *Da Huo Luo Dan*

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ABSTRACT

A modification of *Da Huo Luo Dan* is marketed and prescribed for the treatment of paresis and paralysis according to Traditional Chinese Veterinary Medicine principles. The concentrations of essential nutrients and heavy metals within the formula have not been previously reported and could exert physiologic effects. The present investigation employed standard analyses to quantify basic nutrients and inductively-coupled plasma (ICP) spectrometry to determine mineral and heavy metal concentrations in this herbal formula. The results suggest that typical doses of modified *Da Huo Luo Dan* are unlikely to significantly contribute to the macronutrient intake of patients. High mean as-fed (AF) concentrations of aluminum (1,410 mg/kg), barium (1,570 mg/kg), boron (780 mg/kg), and iron (1,590 mg/kg) were identified but are unlikely to cause toxicity. Low levels of cadmium and lead (1.0 mg/kg and 3.8 mg/kg AF) were detected and could represent markers of environmental or processing contamination. Differences were detected between batch lots from the same manufacturer, which requires future study and which could influence the safety and efficacy of the product.

Key words: *Da Huo Luo Dan*, Chinese herbal medicine, paralysis, rehabilitation, heavy metals, ICP, nutrition

ABBREVIATIONS

ICP	Inductively-coupled plasma
DM	Dry matter
AF	As fed
MTL	Maximum tolerable level

Chinese herbal medications are regarded as safe by many TCVM practitioners but the basic nutritional and mineral composition of only the most common veterinary formulas have been previously published.[1] Such compounds, if present in high concentrations, could theoretically contribute to both the potential toxicity of herbal formulas as well as to the mechanism of action. A veterinary specific modification of *Da Huo Luo Dan* is administered by practitioners and contains the neuroexcitatory alkaloids strychnine and brucine at levels below clinical toxicity when given at the recommended dose.[2] The formula is marketed and recommended for the treatment of paresis and paralysis in both large and small animals.[3] The purpose of this study was to quantify the concentrations of essential and potentially toxic nutrients and metals within this herbal formula. It was hypothesized that the formula would not contain clinically significant concentrations of nutrients and contaminant metals when prescribed at the recommended dose.

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MATERIALS AND METHODS

Two batches of a commercially available modified *Da Huo Luo Dan* were obtained from the manufacturer as a dried mixed herbal powder for analysis (Table 1). A sample of 25 grams obtained from the combination formula was submitted to a commercial forage laboratory for proximate analyses,[a] and a similar amount was provided to a different laboratory for mineral and heavy metal analysis using inductively-coupled plasma spectrometry.[b] Samples were analyzed in duplicates and values are reported as the mean of the two values if the difference between sample results was no greater than ten percent.

RESULTS

The proximate and mineral analyses of each sample of the combination formula are reported (Tables 2 and 3). The two lots varied in composition. The second batch lot, for example, contained greater than ten percent more crude protein, fat, and energy as compared to the first, whereas the first lot contained greater than ten percent more lignin and moisture. The ash content of the first sample was nearly double that of the second, and notable comparative elevations in aluminum, boron, barium and iron were observed. Lead and cadmium were detected in both samples, but other possibly toxic elements such as arsenic and mercury were below detectable limits. Macrominerals were present on both the proximate and mineral analyses, and similar values were reported

between the two laboratories with a percent difference no greater than 10 percent and a mean difference of 6.5%.

DISCUSSION

Chinese herbs contain essential nutrients that are likely to vary between formulas. The calcium and sodium content of this formula was higher than the median reported for other Chinese herbal formulas prescribed by veterinarians whereas the potassium content was lower.[1] The formula was higher in fat and lower in non-fiber carbohydrate than other products which may be due to the inclusion of animal tissue such as *Di Long* (earthworm) and *Quan Xie* (scorpion). Differences were identified between two batches of the formula; for instance, ash was much higher in the first sample than in the second sample, which was also reflected in individual mineral concentrations. One batch contained 2.9 g/kg AF sodium and another 1.2 g/kg AF, and this difference was not explained by the different moisture contents of the two batches (8.9% vs. 6.5%, respectively). Nevertheless, the amounts detected in either batch are unlikely to substantially contribute to daily nutrient requirements.

The maximum-labeled dose of this formula is 220 mg/kg BW daily.[3] A hypothetical inactive 45-kg pet dog receiving this amount of herbal powder daily would receive 1.3 g DM protein and about 80 mg DM Ca, or approximately 2.4% of the National Research Council's recommended allowance of protein and 3.7% of the calcium allowance for adult dogs.[4] The nutrients in this Chinese herbal formula are therefore unlikely to contribute to the patient's essential nutrient requirements unless fed long-term and with high daily doses, consistent with the results of a prior investigation.[1]

Trace mineral and heavy metal contamination could have potentially toxic effects in high doses, and a comparison of maximum tolerable levels in a dog and the dog's intake when receiving the maximum dose is presented in Table 4. The aluminum concentration was higher than previously reported in Chinese herbal formulas marketed for human consumption and in other veterinary-specific formulas.[1,5] Veterinary formulas were found to contain a median of 380 mg/kg DM and a maximum of 940 mg/kg DM in a previous study, whereas this formula contained a mean value of 1,500 mg/kg DM. The reason for elevated aluminum in this and other Chinese veterinary herbal formulas is unclear but could be related to increased use of aluminum storage containers or processing equipment, adulteration, or the use of aluminum containing preservatives.[1] A dietary maximum tolerable level (MTL) is not established for small animals although a value of 1,000 mg/kg has been suggested for horses and cattle.[6] Horses and dogs tolerate high doses of aluminum unless renal function is impaired, and this formula is unlikely to be administered to animals with concurrent severe azotemia.[7,8] In addition, the labeled dose of *Da Huo Luo Dan* would only be a small portion of an animal's diet, so aluminum toxicity is further unlikely unless the remainder of the diet also contained a large

Table 1: Labeled ingredients in Modified *Da Huo Luo Dan*[3]

Pin Yin	Common name
Ba Ji Tian	Morinda
Bu Gu Zhi	Psoralea
Chi Shao	Paeonia
Chuan Xiong	Ligusticum
Di Long	Pheretima
Du Zhong	Eucommia
Fu Zi (Shu)	Aconite
Gan Cao	Glycyrrhiza
Gu Sui Bu	Drynaria
Hong Hua	Carthamus
Huang Qi	Astragalus
Ma Qian Zi	Strychnos
Mo Yao	Myrrh
Quan Xie	Buthus
Ru Xiang	Olibanum
(Tian) San Qi	Notoginseng
Wu Yao	Lindera
Xu Duan	Dipsacus
Xue Jie	Draconis
Chuan Niu Xi	Cyathula
Dang gui	Angelica

Table 2: Mean Proximate Analysis of 2 samples of Modified *Da Huo Luo Dan* (% dry matter)

Nutrient	Sample 1	Sample 2	Difference (%)
Crude protein	13.2	15.3	14.7
Acid detergent fiber	26.1	23.1	12.2
Neutral detergent fiber	34.9	33.7	3.5
Lignin	9.9	8.9	10.6
Nonfiber carbohydrates	32.9	34.2	3.9
Crude fat	7.9	12.0	41.2
Ash	14.7	8.1	57.9
Sulfur	0.34	0.3	12.5
Moisture (as fed)	8.9	6.5	31.2
Kcal/g DM (calculated)	2.3	2.8	19.6

concentration of aluminum.

The barium concentration of the analyzed herbal samples (1,570 mg/kg DM) was above the MTL suggested for rodents (250 mg/kg DM) and for horses (100 mg/kg DM). Barium is present naturally as $BaSO_4$ and $BaCO_3$, both of which have poor water solubility. Manufactured salts such as $BaCl_2$ are more soluble and would be expected to have greater bioavailability.[6] Speciation of the high concentration of barium was not performed in this study. Humans tolerate levels of barium sulfate up to 1 g/kg/day.[6] Therefore, dogs receiving the herbal supplement at even three times the labeled dose would be unlikely to show signs of barium toxicity if barium is present as the sulfate form. Additional research is warranted to determine the source and form of the barium identified in this herbal formula.

Boron was identified in one batch of the herbal formula in a concentration exceeding the MTL of 150 mg/kg DM suggested for ruminants, swine, horses, and rodents. Animals may require low doses of boron,

Table 3: Mean Mineral and Heavy Metal Content of Modified *Da Huo Luo Dan*

Element	Sample 1		Sample 2		Lab Difference (%)	Sample Difference (%)
g/kg as fed	**Lab 1**	**Lab 2**	**Lab 1**	**Lab 2**		
Calcium	8.8	8.2	7.7	7.0	8.3	14.5
Phosphorus	2.0	2.1	2.3	2.4	4.6	13.6
Magnesium	2.3	2.4	2.0	2.0	2.1	16.1
Potassium	9.1	8.4	8.2	7.5	8.5	10.8
Sodium	2.8	3.0	1.1	1.2	7.8	86.4
mg/kg as fed						
Aluminum		1760		1050		50.5
Antimony		<5.0		<5.0		0
Arsenic		<2.5		<2.5		0
Barium		1750		1390		22.9
Boron		1530		21.9		194.4
Cadmium		0.9		1.0		10.5
Chromium		6.6		3.4		64.0
Cobalt		1.0		0.7		35.3
Copper	11	13	14	16	15.0	22.2
Iron	1940	2060	1110	1230	8.1	52.4
Lead		4.2		3.3		24.0
Manganese	112	109	106	101	3.8	6.5
Mercury		<10		<10		0
Molybdenum	<1	<1	<1	<1	0	0
Selenium		<10		<10		0
Thallium		<12.5		<12.5		0
Zinc	39	42	51	57	9.3	28.6

Table 4: A Comparison of Maximum Tolerable Levels of Minerals and a 45 kg Dog's Intake at the Maximum Labeled Dose

Element	Maximum Tolerable Level (mg/day)	Hypothetical Intake (mg/day)
Aluminum	400	17
Barium	40	16
Boron	60	15
Iron	Not reported	17
Cadmium	4	0.01
Lead	20	0.04

and plants are known to concentrate the element which is found in nature complexed to oxygen or as a borate. Boron containing compounds are absorbed and excreted in the urine without significant long-term storage, and boron supplementation for osteoarthritis at less than 0.1 mg/kg BW has been reported in dogs, cattle, and horses.[9] Nausea was observed in dogs receiving 750-2500 mg/kg DM of boron as borax or boric acid.[6] A 45 kg dog would need to ingest about 300 mg of boron to cause nausea, and a dog of this size receiving the labeled dose of this herbal product would consume only 15 mg of boron daily, an amount unlikely to cause acute toxicity. Synthesized boron containing products and natural plant or soil boron could explain the elevated levels. Boron is utilized in the manufacture of insecticides, which could theoretically contaminate the constituent herbs within the formula.

Iron was higher in this formula than in the 400 mg/kg DM median reported in an earlier study of Chinese veterinary herbal products.[1] It also exceeded the MTL for rodents and horses and may contribute to the dietary iron intake but this depends on its chemical form and bioavailability which were unknown in this investigation.[4] The mean iron content of 1,700 mg/kg DM would provide 17 mg of iron daily, or 140 percent of the recommended daily allowance to the hypothetical 45 kg dog described in previous examples. No safe upper limit is reported for dogs or cats, and many animals consume substantially more iron in an available form on a daily basis.

Cadmium and lead are two minerals implicated in the contamination of Chinese agricultural products and these could produce toxicity at comparatively low concentrations. The cadmium concentration was approximately 1 mg/kg DM, whereas the mean and median from a number of Chinese herbal products for humans (n=64) was 0.6 mg/kg and 0.1 mg/kg DM, respectively.[10-15] Adult beagles displayed no adverse effects when fed diets containing 10 mg Cd/kg DM for eight years.[6] A large dog receiving this formula would consume 0.25% of this amount.

Lead was detected at a level of approximately 4 mg/kg DM, higher than the level detected in other veterinary combination formulas (n=14) and the median of 0.8 mg/kg DM in previously tested human patent formulas (n=65).[1,10-16] The higher than expected lead content of this particular formula deserves attention, but toxicity is unlikely unless the rest of the diet is also high in lead. Moreover, adult dogs tolerated feeding studies with up to 50 mg Pb/kg diet administered for two years.[6] A large dog receiving this herbal combination would consume 0.2% of this amount. Pb could be higher in this formula due to soil contamination, which has occurred in parts of China, and subsequent plant uptake which is dependent on soil conditions, the amount of contamination, and the plant type.[17-18] Additional testing of each constituent herb could better document the source of the Cd and Pb concentrations detected in the combination formula.

Several mineral concentrations were obtained from two different laboratories using similar techniques. The maximum inter-laboratory difference was ≤10% of the mean for Ca, P, Mg, K, Na, Fe, Mn, and Mo and <20% for Zn and Cu but these values were small (<60 mg/kg) and reported with only 2 significant figures. Therefore, inter-laboratory differences were likely not significant which suggests adequate quality control at both facilities.

The present investigation is subject to several limitations. Only two batches of modified *Da Huo Luo Dan* were available for testing, and substantial differences were noted in the DM concentration of potentially toxic minerals between the two batches. The inter-batch sample difference was >30% of the mean for Na, Al, B, Cr, Co, and Fe. The boron concentration was dramatically different: 1,700 mg/kg DM as opposed to 23 mg/kg DM. Additional samples would have further clarified the extent of the observed inter-batch variability. The variability of plant-based foods and herbs are well documented, and the results of this investigation are consistent with a previous veterinary study which examined differences between single herbs from different suppliers.[1] The detected difference may not be functionally or clinically significant, and the reasons for observed findings could include seasonal variation, intentional or inadvertent adulteration with chemicals or fertilizers, regional changes presumably due to soil or water conditions, incorrect herb identification, and manufacturing processes.[19-21] Consequently, the variation from this small number of samples should be interpreted with caution. Sample size was limited based on the long shelf life of the product from the manufacturer, and on the product being mixed in large batches from which portions were sold over a considerable period of time. The study is also complicated as the commercial laboratories utilized were not under the direct control of the investigator. Moreover, some potentially toxic metals, e.g. mercury and arsenic, were analyzed for which the minimum value that could be detected was above the potentially toxic level; mercury, for example, could not be detected at <10 mg/kg whereas a level around 1 mg/kg could be significant.

A modification of *Da Huo Luo Dan* marketed for veterinary use contains both essential nutrients and potentially harmful metals in quantities unlikely to produce clinically relevant toxicities if fed according to label instructions. However, significant inter-batch variation was detected in macronutrients and in minerals, which may suggest that concentrations of bioactive components of the herbs also vary. Further studies are warranted to analyze the composition of a larger number of veterinary herbal products and to better examine the inter-batch differences between herbs.

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FOOTNOTES

a. Ration Balancer Plus, Dairy One Forage Laboratory, Ithaca, New York
b. Toxic Element Panel and GC-MS toxicology screen, Diagnostic Center for Population and Animal Health, Michigan State University, Lansing, Michigan

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